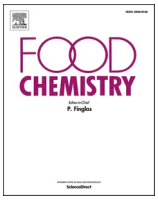
Food Chemistry 411 (2023) 135491

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem



Nutritional, chemical and antioxidant evaluation of Armuña lentil (*Lens culinaris* spp): Influence of season and soil

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ARTICLE INFO

Keywords:
Lens culinaris
Armuña lentil
PGI
Nutritional value
Chemical composition
Bioactive properties

ABSTRACT

Lentils (*Lens culinaris* spp.) are a type of edible pulse consumed and produced worldwide; they are known for their valuable nutritional assets. The nutritional and chemical profiles of 34 Armuña lentil samples were assessed together with their antioxidant capacity. In addition, the influence of both the climatic conditions during the growing season and the soil type in which they grow (Luvisol and Cambisol) on nutritional and chemical profiles was also evaluated. Our results showed large amounts of valuable nutrients, such as carbohydrates, of which approximately 47.06 % and 29.11 % consist of fibers and starch respectively and significant amounts of proteins (20.47 to 25.56 g/100 g fw) and ashes. Sucrose stood out as the main free sugar in this variety, and oxalic and γ-tocopherol as the main organic acid and tocopherol isoform respectively. Fatty acid assessment showed the prevalence of PUFAs (45.3 to 63.7 %). A high antioxidant capacity (TBARS and OxHLIA) was also observed. Our results indicate that the growing season has a significant impact on the major nutrients in lentils such as the concentration of fat, ashes, fibers, and fructose and to a lesser extent proteins and sucrose. In addition, the two different soil types in this study do not seem to affect any of the parameters analyzed.

1. Introduction

In recent years, the acknowledgment of food legumes as important influences on the development of sustainable agriculture and the establishment of improved food security as a result of their cultivation has spread throughout the world (Khazaei et al., 2019). Despite scientifically proven evidence of the benefits of their consumption as part of a daily diet, their consumption is particularly low in some developed countries. This is generally due to different cultural eating habits, low sensory acceptance of this type of food, and the unavailability of other processed leguminous food products (Baik & Han, 2012).

Pulses, the dry edible seeds of the legume family, are considered as staple foods worldwide partly owing to their exceptional chemical and nutritional profiles (Chelladurai & Erkinbaev, 2020). As part of an ancient culture, lentils (*Lens culinaris* spp.) are grown in>70 countries and stand out for being a fast-cooking pulse consumed worldwide and appreciated for their richness in proteins, dietary fibers, complex carbohydrates, and essential micronutrients such as iron, zinc, and the vitamin B complex (Khazaei et al., 2017; Nosworthy et al., 2017). In addition to their outstanding nutritional properties, lentil seeds have a high antioxidant capacity compared to other grain legume species, given the presence of specific phenolic compounds in their composition (Grela et al., 2017). Consequently, over the years lentil consumption has become associated with certain health benefits and is able to prevent and/or control certain cardiovascular diseases, type II diabetes, various types of cancer, and obesity, among other conditions (Pistollato et al., 2015; Rizkalla, Bellisle, & Slama, 2002). According to the Food and Agriculture Organization (FAO), these characteristics as a whole are responsible for the growing importance of lentils as a staple food and their reflection of good eating practices. They show the highest growth rate in global consumption compared with other more common pulses which are more frequently consumed (Szczebyło, Halicka, Jackowska, & Rejman, 2019).

The production of lentils and their chemical composition are largely

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https://doi.org/10.1016/j.foodchem.2023.135491
Received 17 September 2022; Received in revised form 11 January 2023; Accepted 12 January 2023
Available online 18 January 2023

influenced by their resistance to biotic and abiotic stresses, which are conditioned not only by their variety but also by the climatic conditions and the composition of the soils in which they grow (Harumi Lyda et al., 2019). Lentils are usually sown in the fall and harvested at the end of a hot dry summer; a delay in this process results in losses in both quality and quantity parameters, mainly owing to breaks, lodging, loss of pods, disease, and moisture content (Chelladurai & Erkinbaev, 2020). The outcomes deriving from different environmental stresses to which plants are exposed may diverge according to the intensity and duration of the same, which has both beneficial and harmful effects for the plant (Khazaei et al., 2019). In addition, among the edaphic influences, the availability of plant nutrients is of supreme importance. The growth, yield, chemical and nutritional composition of lentils are influenced by the presence of different micronutrients in the soil (Karan & Singh, 2014). These and other features have encouraged research into a larger number of lentil varieties cultivated in different countries and exposed to different edaphic factors. Spain is the major European producer of lentils and together with France accounts for 70 % of total European production. However, although Spain increased its annual production to 46.4 thousand tons by 2020 according to data from the Spanish Ministry of Agriculture, Fisheries and Food (MAPA, 2020), importing lentils is still necessary to meet the demand for national consumption. In Spain, lentil production is associated with climatic change as they grow under non-irrigated conditions. The region of La Armuña, in the north of the province of Salamanca, belongs to a Protected Geographical Indication (PGI) which is characterized by low precipitation, long cold winters, and hot dry summers. The lentils grown in this PGI belong to the Armuña variety, which presents large grains (5 to 7 mm) weighing between 5 and 8 g (Revilla et al., 2019) and light green color with a blotched pattern (Plaza, Remedios Morales-Corts, Pérez-Sánchez, Revilla, & Vivar-Quintana, 2021), and a characteristic texture, which is why it is highly appreciated by consumers. Its average yield is around 423 kg/ha as the flowering of this variety is greatly affected by environmental conditions (Rubiales, Moral, & Flores, 2021). In terms of nutritional composition, it stands out for its high protein content (>24 %) and its low fat (<0.7 %) and carbohydrate (<60 %) content compared to other Spanish lentil varieties (Plaza et al., 2021). Starch is the major component of its composition with a low in vitro digestibility rate. (Gutierrez, 2018). In addition, it shows a high content of fiber, calcium and iron (Dueñas et al., 2016).

This study aims to provide a full evaluation of the nutritional and phytochemical profiles and also the antioxidant capacity of different samples of Armuña lentils grown in two different soil types (Luvisol and Cambisol) in different years (2018 and 2019), so as to contribute to the characterization of this variety and the potential influence of environmental and edaphic factors on its composition. The characterization of local lentil varieties contributes to the development of sustainable food systems as outlined in the 2030 Agenda. Local varieties are better adapted to their agroecological and production systems and also to the preferences of end consumers. Understanding the influence of soil and climatic conditions on these varieties is relevant in the context of food security, rural development and the resilience of farming communities as stated by the FAO (2019).

2. Materials and methods

2.1. Samples

A total of 34 samples of Armuña lentils (*Lens culinaris* spp.) grown in two different years (2018 and 2019) were analyzed. All samples examined were provided by the Regulatory Council of the Lenteja de la Armuña Protected Geographic Indication and collected from 16 municipalities in the district of Armuña, in the north of the province of Salamanca (32 samples, from the "Lenteja de La Armuña" PGI) and in the province of Guadalajara (2 samples, not from a PGI). In addition, two different soil types were considered in this study; these were established according to the FAO classification system as Cambisol (calcaric and chromic) and Luvisol (calcic and vertic). The soil classification for different sowing zones was based on the maps drawn up by the Institute of Natural Resources and Agrobiology of Salamanca (IRNASA-CSIC). Data regarding the environmental conditions (i.e., annual rainfall, humidity, and solarization, among others), were recorded at a weather station near the experimental site (Aldearrubia, Salamanca). The average annual temperatures were 9.25 °C (a maximum of 26 and a minimum of −3.06 °C) and 9.98 °C (a maximum of 26.13 and a minimum of −2.64 °C) for the 2018 and 2019 harvests respectively. For the same years precipitation ranged from 358 mm to 176 mm, respectively.

Samples were cleaned to remove foreign material and damaged seeds before being transferred to the laboratory. Prior to the analytical assessment, samples were grounded in a Foss Knifetec™ 1095 mill at a controlled temperature (20 °C) and stored until further analysis.

2.2. Chemical composition

2.2.1. Proximate composition and energetic value

The nutritional content (protein, carbohydrates, and ash) of the lentils was studied by using the AOAC procedures (AOAC, 2016). The macro-Kjeldahl method was employed to determine the crude protein content (N × 6.25); Soxhlet apparatus was used to estimate crude fat by extracting a known weight of the samples with petroleum ether; the ash content was determined by incineration of the samples at 550 ± 10 °C; and dietary fibers were assessed following an enzymatic–gravimetric method involving enzymatic digestions with α-amylase, protease and amyl glucosidase. Subsequently the protein and ash contents were analyzed in the residues digested according to the methods mentioned above. Samples were analyzed in duplicate and the results expressed in relative percentages according to the equation: % of total dietary fiber = [(R – P - A) / SW] × 100 in which R is the average residue, P the average proteins, A the average ashes, and SW the average weight of the samples.

The starch content was determined according to the methods described by Alajaji and El-Adawy (2006) as reducing sugars after complete acid hydrolysis. The total amount of carbohydrates and total energy were assessed respectively by differences ((100 – (Moisture + Ash + Protein + Total fat + Total fiber) and according to the following equation: energy (kcal/100 g fresh weight (fw)) = 4 × (g protein + g carbohydrates) + 9 × (g fat).

2.2.2. Free sugars

High-performance liquid chromatography coupled to a refraction index detector (HPLC-RI) was used to determine free sugars after an extraction procedure previously described by Spréa et al. (2020), with melezitose being utilized as an internal standard (IS). Identification and quantification were carried out by comparing with authentic standard retention times and using the IS method and the calibration curves created from the standards quoted respectively. Free sugar concentrations were expressed in g per 100 g of fw. The standards used were fructose, sucrose, glucose trehalose and raffinose from Sigma-Aldrich (St. Louis, MO, USA)).

2.2.3. Organic acids

The organic acids were assessed by following the procedure previously described and optimized by Pereira, Barros, Carvalho, and Ferreira (2013). The analysis was performed using a Shimadzu 20A series UFLC and detection was carried out in a PDA, using 215 nm as the preferred wavelength. The organic acids found were quantified by comparing the area of the peaks with calibration curves obtained from the commercial standards of oxalic, quinic, malic, ascorbic, shikimic, citric, succinic, and fumaric acids purchased from Sigma-Aldrich (St. Louis, MO, USA). For quantitative analysis, the calibration curves were determined from different standard compounds. The results were expressed in g per 100 g of fw.

2.2.4. Tocopherols

The tocopherols were determined by following the procedure previously described by Spréa et al. (2020). The analysis was performed using an HPLC system (as described above) and a fluorescence detector (FP-2020; Jasco) programmed for excitation at 290 nm and emission at 330 nm. The compounds were identified by chromatographic comparisons with authentic standards. Quantification was based on the fluorescence signal response of each standard using the IS (tocol) method and the calibration curves obtained from the commercial standards of α, β, and γ-tocopherol purchased from Matreya (PA, USA). The results were expressed in mg per 100 g of fw.

2.2.5. Fatty acids

Fatty acid methyl esters (FAME) were studied after transesterification of the lipid fraction obtained by means of Soxhlet extraction as described above (Spréa et al., 2020) and determined by gas–liquid chromatography with flame ionization detection, using a YOUNG IN Crhomass 6500 GC System instrument equipped with a *split/spitless* injector, a flame ionization detector (FID), and a Zebron-Fame column. The fatty acids were identified and quantified by comparing the relative retention times of FAME peaks with samples of commercial standards (standard mixture 47885-U, Sigma, St. Louis, USA). The results were recorded and processed using the Clarity DataApex 4.0 Software (Prague, Czech Republic) and expressed in relative percentages of each fatty acid.

2.3. Antioxidant activity evaluation

2.3.1. Preparation of extracts

For the preparation of the extracts 3 g of ground lentils were dissolved in 100 mL of water and boiled for 10 min. The mixture was then filtered through Whatman paper No. 4. The resulting extracts were then frozen and lyophilized so as to obtain a dried extract (FreeZone 4.5, Labconco, Kansas City, MO, USA).

2.3.2. Thiobarbituric acid reactive substances (TBARS)

For the TBARS assay the lyophilized decoction extracts were dissolved in distilled water and subjected to dilutions from 0.01953 to 0.3125 mg/mL. Lipid peroxidation inhibition in porcine brain homogenates was evaluated by the decrease in TBARS. Brains were obtained from pig (Sus scrofa Linnaeus*)*, dissected and homogenized in ice-cold Tris–HCl buffer (pH 7.4, 20 mM) and centrifuged at 3000 g for 10 min. The color intensity of the malondialdehyde-thiobarbituric acid (MDA–TBA) complex was measured by its absorbance at 532 nm; the inhibition ratio (%) was calculated by using the following formula: [(A − B)/A] × 100 %, in which A and B refer to the absorbance of the control and the sample solutions respectively (Roriz, Barros, Carvalho, Santos-Buelga, & Ferreira, 2014). The results were expressed in EC_{50} values (μg/mL with the sample concentration accounting for 50 % of the antioxidant activity). Trolox was used as a positive control.

2.3.3. Oxidative hemolysis inhibition assay (OxHLIA)

The anti-hemolytic activity of the lyophilized decoction extracts was evaluated using the OxHLIA assay as described in detail by Lockowandt et al. (2019). An erythrocyte solution (2.8 %, *v/v;* 200 μL) was mixed with 400 μL of either extract solution (0.0938–3 mg/mL PBS), PBS (control), or water (for complete hemolysis). Erythrocytes were obtained from sheep male blood following the methodology described in Barros et al. (2008). After pre-incubation at 37 °C for 10 min while shaking, AAPH (200 μL, 160 mM in PBS, from Sigma-Aldrich) was added and the optical density was measured at 690 nm each ~ 10 min in a microplate reader (Bio-Tek Instruments, ELX800) until complete hemolysis. Trolox was used as a positive control. The results were expressed as IC_{50} values (μg/mL) at Δt of 60, which refers to the extract concentration required to keep 50 % of the erythrocyte population intact for 60 min.

2.4. Statistical analysis

Thirty-four samples (100 g of lentils) were analyzed for all the experiments (duplicate) and the antioxidant capacity (triplicate). The results are expressed as mean values ± standard deviation (SD). The differences between samples were analyzed using a one-way analysis of variance (ANOVA) followed by Tukey's significant difference post hoc test with $\alpha = 0.05$ combined with Welch's statistic. The different parameters were correlated with the growing season and the soil by a Pearson 2-tailed significance correlation. This analysis was carried out using the SPSS v. 23.0 program.

3. Results and discussion

3.1. Proximate composition

The nutritional and chemical composition of plants is strongly influenced by the soil composition and climate conditions of the cultivation locations, the harvest season, and different agricultural practices, among other factors (Harumi Lyda et al., 2019). In order to assess whether these variables lead to this type of alterations, the lentils under investigation were collected at different locations in the Armuña region (Salamanca, "Lenteja de La Armuña" PGI) and in the province of Guadalajara (not a PGI). In addition, samples were cultivated in two different soil types (Cambisol and Luvisol) and harvested in different seasons (2018 and 2019).

The proximate results obtained for macronutrients are presented in Table 1**,** with carbohydrates appearing as the main macronutrients (63.7 to 69.8 g/100 g fw) in Armuña lentils. Proteins ranked next with values varying from 20.5 to 25.6 g/100 g fw and there was also an interesting amount of ashes (1.82 to 3.40 g/100 g fw) consonant with valuable amounts of micronutrients characteristic of this group of pulses. Similar results were obtained in our previous study (Liberal et al., 2021) of Pardina lentils from the "Lenteja Tierra de Campos" PGI, in which the nutritional assessment revealed high amounts of carbohydrates (62.2 to 67.8 g/100 g fw), proteins (20.6 to 25.9 g/100 g fw), and ashes (2.1 to 2.8 g/100 g fw). Furthermore, in both studies low fat contents were identified in lentils, which make them an excellent food product to be included in low-calorie diets. A high content of dietary fibers and starch was also found in the Armuña lentils studied with values ranging from 42 to 47.1 % and 19.30 to 29.11 % respectively; no statistically significant differences were observed in these and the above-mentioned parameters.

The growing season showed a much more marked influence on the nutritional composition when compared with the soil type (Table 2), with which only carbohydrates provide a weak correlation. The highest average protein levels were found in Armuña lentils grown in Luvisol soil in 2019 (23.9 g/100 g fw), which were higher than the average values found for both Cambisol soil (23.1 g/100 g fw) and the year 2018 (22.9 and 22.1 g/100 g fw in both soils respectively). The differences observed can be attributed to the lower precipitation rates of 2019 (176 mm) compared with the previous year (358 mm); since then, it has been reported that water shortages and high temperatures often lead to high protein contents (Al-Karaki & Ereifej, 1999; Nikolopoulou, Grigorakis, Stasini, Alexis, & Iliadis, 2006). For the same reasons, higher average fat concentrations were identified in the Armuña lentils harvested in 2019 in both Luvisol and Cambisol soils (0.91 and 0.92 g/100 g fw respectively) compared with the year 2018 (0.65 and 0.69 g/100 g fw). As far as ashes are concerned, despite the fact that data analysis did not show a correlation with soil type (Table 2), the higher average concentrations were identified in Luvisol soil in both the 2018 and 2019 growing seasons (2.4 and 3.1 g/100 g fw), which may be associated with the composition of this type of soil with a mixed clay accumulation sublayer with high levels of available nutrient ions, including calcium, magnesium, sodium and potassium. (Lavkulich & Arocena, 2011). Furthermore, total dietary fibers seem to be moderately influenced by the

Table 1
The proximate composition and energetic value of the Armuña lentils studied (mean ± SD).

Samples	Year	Soil type	Moisture (%)	Fat (g/100 g fw)	Proteins (g/100 g fw)	Ash (g/100 g fw)	Fibers (%)	Starch (%)	Total Carbohydrates (g/100 g fw)	Energy (kcal/100 g fw)
1	2018	Luvisol	6.37 ± 0.01^{mn}	0.63 ± 0.03^{lmn}	22.6 ± 0.3^{gh}	2.59 ± 0.07^{kl}	23.33 ± 0.01^{m}	44.3 ± 0.3^{hij}	67.8 ± 0.1^{cdefg}	367.3 ± 0.1^{bcd}
2			6.65 ± 0.01^{ijk}	0.64 ± 0.02^{jklmn}	22.45 ± 0.04^{gh}	2.27 ± 0.01^{lm}	23.65 ± 0.01^{l}	43.9 ± 0.8^{ijkl}	68 ± 1^{bcde}	367.54 ± 0.07^{de}
3			7.76 ± 0.01^{ef}	0.66 ± 0.03^{ijklmn}	22.5 ± 0.4^{gh}	2.16 ± 0.05^{mno}	20.11 ± 0.01^{z}	45.6 ± 0.6^{cdef}	66.9 ± 0.3^{ijk}	363.6 ± 0.2^{j}
4			8.41 ± 0.01^{b}	0.64 ± 0.06^{lmn}	23.07 ± 0.32^{fg}	2.52 ± 0.02^{fghij}	22.23 ± 0.01^{s}	46.3 ± 0.7^{abcde}	65.4 ± 0.3^{nopq}	359.5 ± 0.3^{p}
5			4.75 ± 0.01^{q}	0.72 ± 0.01^{hijklm}	23.4 ± 0.7^{ef}	2.4 ± 0.1^{hij}	21.56 ± 0.01^{x}	42.9 ± 0.7^{lmnopq}	68.7 ± 0.5^{bcde}	374.8 ± 0.2^{a}
6			7.99 ± 0.01^{cd}	0.69 ± 0.01^{fghij}	24.5 ± 0.6^{b}	2.8 ± 0.2^{fghi}	19.30 ± 0.01^{z}	44.1 ± 0.7^{ijk}	64.1 ± 0.3^{qr}	360.4 ± 0.6^{mn}
7			7.79 ± 0.01^{ef}	0.63 ± 0.06^{lmn}	24.2 ± 0.4^{bcd}	2.64 ± 0.04^{efgh}	20.15 ± 0.01^{z}	43.2 ± 0.7^{klmno}	64.8 ± 0.2^{opq}	361.4 ± 0.3^{mn}
8			7.69 ± 0.01^{f}	0.65 ± 0.05^{mn}	22.4 ± 0.8^{gh}	1.82 ± 0.08^{p}	21.67 ± 0.01^{w}	44.0 ± 0.8^{ijk}	67.4 ± 0.6^{fghij}	365.2 ± 0.4^{hi}
9			9.27 ± 0.01^{a}	0.61 ± 0.09^{klmn}	21.1 ± 0.9^{kl}	2.27 ± 0.08^{lm}	23.78 ± 0.01^{k}	47 ± 1^{a}	66.8 ± 0.6^{jkl}	356.9 ± 0.6^{p}
10		Cambisol	7.19 ± 0.01^{gh}	0.70 ± 0.02^{ijklmn}	22.6 ± 0.1^{gh}	2.22 ± 0.07^{lm}	22.52 ± 0.01^{q}	47.1 ± 0.7^{ab}	67.29 ± 0.08^{ghij}	365.9 ± 0.1^{gh}
11			7.19 ± 0.01^{gh}	0.67 ± 0.05^{ijklm}	21.4 ± 0.6^{jk}	1.83 ± 0.09^{p}	20.11 ± 0.01^{z}	42.0 ± 0.7^{pq}	68.9 ± 0.5^{ab}	367.29 ± 0.06^{bcd}
12			7.86 ± 0.01^{de}	0.78 ± 0.03^{fghijk}	21.7 ± 0.3^{ij}	2.30 ± 0.08^{lm}	20.27 ± 0.01^{z}	44.2 ± 0.2^{ij}	67.3 ± 0.2^{defghi}	363.2 ± 0.3^{jkl}
13			7.11 ± 0.01^{h}	0.77 ± 0.03^{ghijkl}	23.8 ± 0.4^{cde}	1.97 ± 0.04^{o}	20.33 ± 0.01^{z}	43.8 ± 0.6^{ijklm}	66.4 ± 0.3^{defghi}	367.5 ± 0.2^{de}
14			8.51 ± 0.01^{b}	0.62 ± 0.05^{mn}	21 ± 1^{kl}	2.20 ± 0.09^{mno}	20.90 ± 0.01^{z}	45.3 ± 0.4^{efgh}	67.7 ± 0.6^{efghi}	360.2 ± 0.4^{n}
15			7.19 ± 0.01^{gh}	0.59 ± 0.02^{mn}	22.2 ± 0.4^{hi}	2.52 ± 0.08^{jk}	21.99 ± 0.01^{u}	46.2 ± 0.3^{bcde}	67.6 ± 0.2^{fghij}	364.1 ± 0.2^{ij}
16		Not a PGI	6.63 ± 0.01^{ijk}	0.55 ± 0.07^{n}	22.9 ± 0.4^{fg}	2.05 ± 0.08^{no}	23.90 ± 0.01^{h}	44.5 ± 0.4^{ghi}	67.9 ± 0.4^{cdefgh}	368.01 ± 0.01^{cd}
17			7.33 ± 0.01^{g}	0.71 ± 0.01^{efghi}	21.2 ± 0.1^{jk}	1.99 ± 0.04^{no}	22.47 ± 0.01^{r}	46.4 ± 0.4^{abcd}	68.82 ± 0.09^{bc}	366.3 ± 0.2^{fg}
18	2019	Luvisol	6.01 ± 0.01^{o}	1.02 ± 0.04^{b}	25.3 ± 0.8^{a}	3.08 ± 0.02^{cd}	29.11 ± 0.01^{a}	42.4 ± 0.7^{nopq}	64.6 ± 0.6^{opq}	368.74 ± 0.09^{bc}
19			6.31 ± 0.01^{n}	0.92 ± 0.01^{cdef}	26 ± 1^{a}	2.97 ± 0.03^{cde}	22.82 ± 0.01^{o}	44.7 ± 0.3^{fghi}	64.3 ± 0.6^{pqr}	367.5 ± 0.04^{def}
20			7.27 ± 0.01^{gh}	0.93 ± 0.04^{bcd}	25.2 ± 0.3^{a}	2.96 ± 0.04^{de}	26.38 ± 0.01^{c}	45.4 ± 0.4^{defg}	63.7 ± 0.2^{r}	363.7 ± 0.2^{j}
21			6.73 ± 0.01^{ij}	0.93 ± 0.05^{bc}	23.8 ± 0.6^{cde}	2.67 ± 0.03^{ghij}	21.98 ± 0.01^{v}	44.75 ± 0.08^{fghi}	65.9 ± 0.3^{lm}	367.07 ± 0.09^{de}
22			7.96 ± 0.01^{cd}	0.90 ± 0.07^{cdef}	22.6 ± 0.7^{gh}	2.28 ± 0.04^{lm}	23.84 ± 0.01^{i}	46.6 ± 0.4^{abc}	66.3 ± 0.5^{klm}	363.6 ± 0.4^{jk}
23			5.97 ± 0.01^{o}	0.71 ± 0.03^{ijklm}	24.30 ± 0.03^{bc}	3.14 ± 0.01^{b}	23.20 ± 0.01^{n}	43.3 ± 0.5^{jklmn}	65.89 ± 0.05^{mn}	367.1 ± 0.1^{efg}
24			8.06 ± 0.01^{c}	0.93 ± 0.03^{cdefgh}	21.9 ± 0.3^{m}	3.31 ± 0.05^{ab}	26.17 ± 0.01^{d}	43 ± 1^{nopq}	65.8 ± 0.2^{bcdef}	359.2 ± 0.3^{o}
25			6.41 ± 0.01^{lmn}	0.97 ± 0.09^{bcd}	21.2 ± 0.6^{jk}	3.40 ± 0.04^{b}	24.11 ± 0.01^{g}	43.1 ± 0.8^{klmnop}	68.1 ± 0.3^{bcd}	365.6 ± 0.2^{fgh}
26		Cambisol	6.73 ± 0.01^{ij}	0.43 ± 0.01^{o}	22.8 ± 0.1^{fgh}	2.54 ± 0.06^{lmn}	20.94 ± 0.01^{z}	42 ± 1^{opq}	67.6 ± 0.1^{fghij}	365.0 ± 0.1^{cde}
27			5.60 ± 0.01^{p}	0.91 ± 0.07^{cde}	25.5 ± 0.5^{a}	2.19 ± 0.02^{mno}	25.50 ± 0.01^{e}	42.9 ± 0.5^{lmnopq}	65.8 ± 0.3^{lm}	373.4 ± 0.3^{a}
28			7.88 ± 0.01^{de}	0.78 ± 0.08^{efghi}	24.3 ± 0.4^{cde}	2.27 ± 0.01^{lm}	21.20 ± 0.01^{y}	44.0 ± 0.8^{ijk}	64.8 ± 0.2^{mno}	363.3 ± 0.3^{jk}
29			7.63 ± 0.01^{f}	0.97 ± 0.04^{cdefgh}	21.5 ± 0.8^{jk}	2.95 ± 0.08^{de}	24.16 ± 0.01^{z}	42 ± 1^{q}	67 ± 1^{hijk}	362.55 ± 0.09^{lm}
30			6.57 ± 0.01^{jkl}	1.18 ± 0.07^{a}	22.3 ± 0.5^{ghi}	2.87 ± 0.05^{efg}	22.22 ± 0.01^{r}	44.4 ± 0.4^{ghj}	67.1 ± 0.3^{hijki}	368.1 ± 0.4^{bcd}
31			6.49 ± 0.01^{klm}	1.25 ± 0.08^{a}	24.5 ± 0.2^{b}	2.95 ± 0.06^{efg}	22.77 ± 0.01^{p}	46.3 ± 0.9^{bcde}	64.8 ± 0.2^{nop}	368.5 ± 0.5^{bc}
32			6.75 ± 0.01^{i}	0.89 ± 0.01^{cdefg}	22.5 ± 0.4^{gh}	3.38 ± 0.09^{a}	25.12 ± 0.01^{f}	46.5 ± 0.2^{abc}	66.5 ± 0.3^{nop}	363.9 ± 0.2^{jkl}
33			6.22 ± 0.01^{mn}	0.92 ± 0.04^{cd}	20.5 ± 0.7^{l}	2.59 ± 0.08^{ijk}	20.90 ± 0.01^{z}	43 ± 1^{mnopq}	69.8 ± 0.6^{a}	369.4 ± 0.3^{b}
34			7.77 ± 0.01^{ef}	0.96 ± 0.03^{bcd}	23.62 ± 0.03^{de}	2.88 ± 0.01^{def}	27.26 ± 0.01^{b}	42.3 ± 0.8^{opq}	64.77 ± 0.01^{opq}	362.2 ± 0.1^{kl}

Different letters in the same column show no significant differences between means according to Tukey's HSD test ($p > 0.05$).

Table 2
Correlation coefficients of the chemical composition, year, soil, and year × soil of the Armuña lentils studied.

	Year influence	Soil influence	Year-soil interaction
Moisture (%)	−0.302	−0.010	0.049*
Fat (g per 100 g fw)	0.708****	0.051*	
Proteins (g per 100 g fw)	0.304**	−0.260	
Ash (g per 100 g fw)	0.669***	−0.346	
Fibers (%)	0.528***	−0.113	
Starch (%)	−0.283	0.131*	
Carbohydrates (g per 100 g fw)	−0.370	0.335*	
Energy (kcal per 100 g fw)	0.154*	0.182*	
Frutose (g per 100 g fw)	0.575***	0.114*	
Sucrose (g per 100 g fw)	0.490**	0.131*	
Raffinose (g per 100 g fw)	0.280*	−0.018	
Total Sugars (g per 100 g fw)	0.544***	0.108*	
Oxalic acid (g per 100 g fw)	−0.420	−0.167	
Malic acid (g per 100 g fw)	−0.298	0.043*	
Total organic acids (g per 100 g fw)	−0.419	−0.158	
α-Tocopherol (mg per 100 g fw)	−0.511	−0.215	
γ-Tocopherol (mg per 100 g fw)	−0.186	0.033*	
Total Tocopherols (mg per 100 g fw)	−0.245	0.000*	
SFA (%)	0.053*	−0.286	
MUFA (%)	0.161*	−0.078	
PUFA (%)	0.074*	0.263*	
TBARS	0.623***	−0.039	
OxHLIA	0.574***	−0.061	

*, **, ***, ****Negligible, weak, moderated, and strong correlation respectively.

growing season but unaffected by the soil type, with the higher average percentage being identified in the 2019 growing season (24 %). In contrast, the starch concentration seems not to be influenced by either the soil type or year of production. Previous studies on Spanish lentil cultivars (Plaza et al., 2021) have shown that protein, carbohydrates and fiber content are affected by the growing season. To the best of our knowledge, this is the first study to analyze the influence of soil on the nutritional composition of lentils.

The analysis of variance provided in Table 2 shows that the growing season had a significant effect on major nutrients of Armuña lentils, namely fat, ashes, and fibers. The correlations with proteins and total energy were also lower. In contrast, there does not appear to be a significant correlation between the soil type and the variations verified in the concentrations of the parameters analyzed.

3.2. Free sugars

The presence of free sugars was also assessed, and the results are given in Table 3. In all the Armuña lentil samples analyzed fructose, sucrose and raffinose were identified, with sucrose standing out as the main free sugar in this variety and ranging from 1.41 to 2.17 g/100 g fw. Fructose and raffinose, in their turn, were found to be present in much lower amounts (0.06 to 0.23 and 0.23 to 0.64 g/100 g fw respectively), which together with sucrose and the high resistant starch suggest a low-glycemic index of this lentil variety. No statistically significant variations ($p > 0.05$) were detected between the mean values attained for the compounds found in all samples. The results given partly coincide with those of our previous study (Liberal et al., 2021) in which sucrose and raffinose were also identified in Pardina lentils. Although in this study sucrose was the predominant sugar, it was present in lower concentrations ranging from 0.90 to 1.14 g/100 g fw. Furthermore, fructose was identified in Armuña lentils, which was not verified in the Pardina variety. Johnson et al. (2015), when determining the concentration of low molecular weight carbohydrates in a total of 335 lentil samples from 10

Table 3
Composition in free sugars (g/100 g fw) of the Armuña lentils studied (mean ± SD).

Samples	Year	Soil type	Frutose	Sucrose	Raffinose	Total Sugars
1	2018	Luvisol	0.11 ± 0.01^{l}	1.79 ± 0.07^{n}	0.39 ± 0.03^{jklm}	2.29 ± 0.05^{qr}
2			0.23 ± 0.01^{a}	2.14 ± 0.09^{d}	0.64 ± 0.04^{a}	3.00 ± 0.04^{b}
3			0.16 ± 0.01^{fghi}	1.78 ± 0.07^{n}	0.41 ± 0.01^{hijk}	2.35 ± 0.08^{pq}
4			0.12 ± 0.02^{l}	2.03 ± 0.01^{ghi}	0.40 ± 0.01^{ijkl}	2.55 ± 0.03^{jklm}
5			0.15 ± 0.02^{ghi}	1.94 ± 0.02^{l}	0.45 ± 0.02^{fg}	2.54 ± 0.03^{klmn}
6			0.12 ± 0.04^{kl}	1.94 ± 0.03^{kl}	0.43 ± 0.05^{fg}	2.49 ± 0.06^{lmn}
7			0.12 ± 0.02^{kl}	2.05 ± 0.01^{fgh}	0.43 ± 0.02^{gh}	2.60 ± 0.05^{hijk}
8			0.06 ± 0.01^{m}	1.81 ± 0.03^{mn}	0.43 ± 0.05^{ghi}	2.29 ± 0.08^{qr}
9			0.12 ± 0.02^{jkl}	1.66 ± 0.03^{p}	0.23 ± 0.02^{q}	2.02 ± 0.08^{s}
10		Cambisol	0.09 ± 0.01^{l}	1.41 ± 0.04^{q}	0.34 ± 0.01^{o}	1.84 ± 0.04^{f}
11			0.12 ± 0.02^{jkl}	2.1 ± 0.2^{b}	0.37 ± 0.04^{lmno}	2.6 ± 0.2^{ef}
12			0.15 ± 0.01^{ghi}	2.17 ± 0.09^{bc}	0.36 ± 0.01^{no}	2.68 ± 0.09^{ef}
13			0.11 ± 0.01^{l}	2.05 ± 0.01^{fgh}	0.38 ± 0.01^{klmn}	2.54 ± 0.03^{klmn}
14			0.13 ± 0.01^{jkl}	1.72 ± 0.01^{o}	0.41 ± 0.03^{hij}	2.26 ± 0.02^{r}
15			0.11 ± 0.01^{l}	1.97 ± 0.03^{jkl}	0.34 ± 0.01^{o}	2.42 ± 0.06^{op}
16		Not a PGI	0.14 ± 0.01^{ijk}	2.13 ± 0.05^{de}	0.40 ± 0.05^{ijkl}	2.7 ± 0.1^{fgh}
17			0.14 ± 0.03^{hij}	2.00 ± 0.06^{fgh}	0.42 ± 0.02^{ghij}	2.6 ± 0.1^{hij}
18	2019	Luvisol	0.20 ± 0.04^{bcd}	2.12 ± 0.01^{de}	0.38 ± 0.01^{klmn}	2.70 ± 0.03^{efg}
19			0.20 ± 0.01^{bcd}	2.08 ± 0.03^{ef}	0.29 ± 0.02^{p}	2.57 ± 0.06^{ijkl}
20			0.22 ± 0.02^{ab}	2.34 ± 0.06^{a}	0.52 ± 0.02^{d}	3.08 ± 0.07^{a}
21			0.07 ± 0.01^{m}	2.34 ± 0.02^{a}	0.58 ± 0.04^{bc}	3.00 ± 0.07^{bce}
22			0.19 ± 0.01^{cde}	2.08 ± 0.02^{ef}	0.47 ± 0.04^{ef}	2.75 ± 0.05^{e}
23			0.12 ± 0.02^{jkl}	2.01 ± 0.03^{hij}	0.35 ± 0.04^{o}	2.47 ± 0.01^{no}
24		Cambisol	0.16 ± 0.02^{fghi}	2.09 ± 0.01^{ef}	0.37 ± 0.02^{lmno}	2.62 ± 0.05^{hij}
25			0.20 ± 0.02^{bcd}	2.13 ± 0.05^{de}	0.43 ± 0.01^{gh}	2.76 ± 0.03^{e}
26			0.23 ± 0.07^{a}	1.85 ± 0.07^{m}	0.40 ± 0.06^{ijkl}	2.5 ± 0.2^{mno}
27			0.21 ± 0.01^{abc}	2.12 ± 0.05^{de}	0.37 ± 0.04^{mno}	2.70 ± 0.09^{efg}
28			0.16 ± 0.02^{fghi}	2.35 ± 0.03^{a}	0.42 ± 0.03^{ghij}	2.93 ± 0.08^{cd}
29			0.16 ± 0.01^{fghi}	1.99 ± 0.05^{ijk}	0.43 ± 0.01^{gh}	2.58 ± 0.07^{ijkl}
30			0.17 ± 0.02^{fgh}	2.22 ± 0.04^{b}	0.62 ± 0.01^{ab}	3.00 ± 0.02^{bcd}
31			0.21 ± 0.01^{abc}	2.06 ± 0.03^{fg}	0.62 ± 0.01^{a}	2.88 ± 0.05^{d}
32			0.18 ± 0.01^{def}	2.17 ± 0.08^{cd}	0.56 ± 0.01^{c}	2.91 ± 0.08^{d}
33			0.21 ± 0.01^{abc}	2.34 ± 0.02^{a}	0.48 ± 0.04^{e}	3.04 ± 0.01^{ab}
34			0.17 ± 0.02^{efg}	2.04 ± 0.09^{fgh}	0.42 ± 1.13^{hij}	2.63 ± 0.08^{ghi}

Different letters in the same column show no significant difference between means according to Tukey's HSD test ($p > 0.05$).

locations from 6 countries, were able to identify sorbitol, mannitol, galactinol, sucrose, raffinose + stachyose, verbascose, nystose, and kestose. In this study the concentrations of many of these compounds varied with mean temperatures and the precipitation of the region/country of origin.

The concentration of the free sugars identified was also affected by different climate conditions, since correlations were observed between the 2018 and 2019 harvests. In general, the highest concentrations of fructose, sucrose, and raffinose were observed in the 2019 season, with no apparent influence of the soil type on these parameters. These features combined suggest that in this study the free sugar concentration is only affected by changes in the weather conditions of the cultivation area and not by soil characteristics. In addition, slight differences were observed in the non PGI samples of the province of Guadalajara, with the average sugar concentration being established at intermediate values of the growing seasons analyzed.

3.3. Organic acids and tocopherols

As far as organic acids are concerned, oxalic and malic acids were detected in all the Armuña lentil samples analyzed; the results are expressed in Table 4. Oxalic acid presents as the main compound with concentrations ranging from 0.21 to 2.30 g/100 g fw, followed by smaller amounts of malic acid (0.06 to 0.18 g/100 g fw). The occurrence of organic acids in lentils was previously investigated by ourselves (Liberal et al., 2021) in Pardina lentils from the "Lenteja Tierra de Campos" PGI. The oxalic and shikimic acids were identified in addition to large amounts of citric acid (10.51 to 18.06 g/100 g fw). When comparing the results with those of the present study, only oxalic acid is present in both Pardina and Armuña varieties, which may be explained by different genetic combinations between them. Furthermore, it was shown that the growing season and soil type were not related to the concentration of organic acids. Although the function of oxalic acid in plants is not fully understood, the higher chemical impact of this compound is its strong chelating ability; it operates as an effective antioxidant in some systems (Kayashima & Katayama, 2002). However, oxalic acid was also considered to be an antinutrient given its inhibitory influence on mineral bioavailability and its formative impact on calcium oxalate urinary stones (Massey, Palmer, & Horner, 2001). Malic acid, in its turn, has been described as having a cardioprotective effect in which contiguous mechanisms may be associated with their anti-inflammatory and antiplatelet features (Karn, Chavasit, Kongkachuichai, & Tangsuphoom, 2011).

As for tocopherols, the analysis allowed the detection of α- and γ-tocopherol isoforms in all Armuña lentil samples (Table 4), with γ-tocopherol presenting as the main compound (5.3 to 10.6 mg/100 g fw). In its turn α-tocopherol was the least prevalent isoform with values ranging from 0.27 to 0.97 mg/100 g fw. Compared with our previous study (Liberal et al., 2021), significantly lower concentrations of γ-tocopherol were detected in the Pardina variety (2.5 to 4.3 mg/100 g fw). However, this is the isoform present in greater amounts in this variety in the present study. Furthermore, slight differences were detected between the two varieties regarding the α-tocopherol isoform, with Pardina lentils presenting values in the range of 0.21 to 0.36 mg/100 fw. Different bioactivities have been attributed to the γ-tocopherol isoform, including excellent antioxidant and anti-inflammatory properties; their presence in a given food product is therefore seen as an asset in the prevention of different types of chronic and acute diseases (Tang et al., 2015). No influence of season or soil type was found with any of the organic acids. In the case of the tocopherols, only the concentration of gamma tocopherol showed a negligible correlation with soil type (Table 2).

Table 4
Composition in organic acids and tocopherols of the Armuña lentils studied (mg/100 g fw) (mean ± SD).

Samples	Year	Soil type	Oxalic acid	Malic acid	Total organic acids	α-Tocopherol	γ-Tocopherol	Total Tocopherols
1	2018	Luvisol	2.30 ± 0.04^{a}	0.16 ± 0.01^{c}	2.46 ± 0.03^{a}	0.89 ± 0.01^{b}	7.8 ± 0.4^{hij}	8.7 ± 0.5^{efg}
2			1.97 ± 0.01^{b}	0.14 ± 0.01^{de}	2.11 ± 0.01^{b}	0.61 ± 0.03^{g}	7.3 ± 0.3^{k}	7.9 ± 0.2^{ijkl}
3			1.27 ± 0.01^{h}	0.14 ± 0.01^{de}	1.41 ± 0.01^{h}	0.77 ± 0.02^{d}	7.8 ± 0.1^{h}	8.6 ± 0.2^{fg}
4			1.36 ± 0.06^{g}	0.15 ± 0.01^{cd}	1.51 ± 0.06^{g}	0.83 ± 0.05^{c}	9.3 ± 0.2^{cd}	10.2 ± 0.2^{b}
5			1.77 ± 0.03^{d}	0.15 ± 0.01^{cd}	1.92 ± 0.04^{d}	0.97 ± 0.16^{a}	10.1 ± 0.2^{b}	11.1 ± 0.4^{a}
6			1.54 ± 0.05^{e}	0.14 ± 0.05^{de}	1.68 ± 0.10^{e}	0.52 ± 0.07^{hi}	8.98 ± 0.08^{de}	9.5 ± 0.2^{c}
7			0.82 ± 0.01^{m}	0.07 ± 0.01^{lm}	0.89 ± 0.01^{l}	0.66 ± 0.02^{f}	8.3 ± 0.2^{g}	9.0 ± 0.2^{de}
8			0.38 ± 0.03^{u}	0.10 ± 0.01^{hij}	0.48 ± 0.03^{r}	0.52 ± 0.01^{hi}	5.3 ± 0.6^{n}	5.8 ± 0.6^{o}
9			0.46 ± 0.01^{s}	0.09 ± 0.01^{jk}	0.55 ± 0.01^{q}	0.44 ± 0.01^{jkl}	6.6 ± 0.4^{l}	7.0 ± 0.4^{m}
10		Cambisol	1.90 ± 0.09^{c}	0.16 ± 0.01^{c}	2.06 ± 0.08^{c}	0.77 ± 0.06^{de}	7.4 ± 0.2^{jk}	8.2 ± 0.2^{hij}
11			1.14 ± 0.02^{i}	0.10 ± 0.01^{hij}	1.24 ± 0.02^{i}	0.55 ± 0.08^{h}	8.3 ± 0.2^{g}	8.9 ± 0.3^{def}
12			0.31 ± 0.02^{v}	0.13 ± 0.01^{ef}	0.43 ± 0.03^{s}	0.53 ± 0.09^{hi}	10.6 ± 0.4^{a}	11.1 ± 0.3^{a}
13			0.97 ± 0.04^{k}	0.20 ± 0.02^{a}	1.17 ± 0.06^{j}	0.51 ± 0.02^{hi}	8.6 ± 0.5^{fg}	9.0 ± 0.5^{d}
14			0.41 ± 0.02^{tu}	0.06 ± 0.01^{m}	0.48 ± 0.01^{rs}	0.27 ± 0.02^{q}	6.2 ± 0.7^{m}	6.5 ± 0.7^{n}
15			0.67 ± 0.04^{op}	0.13 ± 0.01^{ef}	0.81 ± 0.04^{m}	0.36 ± 0.01^{mno}	6.60 ± 0.02^{l}	6.96 ± 0.01^{m}
16		Not a PGI	1.75 ± 0.09^{d}	0.15 ± 0.01^{cd}	1.90 ± 0.08^{d}	0.79 ± 0.01^{cd}	7.5 ± 0.1^{hijk}	8.3 ± 0.1^{gh}
17			0.21 ± 0.02^{y}	0.08 ± 0.01^{kl}	0.29 ± 0.01	0.48 ± 0.04^{ij}	8.4 ± 0.3^{g}	8.8 ± 0.4^{def}
18	2019	Luvisol	0.51 ± 0.01^{qr}	0.10 ± 0.01^{hij}	0.62 ± 0.02^{p}	0.38 ± 0.01^{mno}	7.5 ± 0.2^{ijk}	7.8 ± 0.2^{jkl}
19			0.90 ± 0.01^{l}	0.11 ± 0.03^{gh}	1.01 ± 0.04^{k}	0.29 ± 0.03^{pq}	6.16 ± 0.06^{m}	6.46 ± 0.08^{n}
20			0.44 ± 0.05st	0.08 ± 0.01^{kl}	0.52 ± 0.06^{qr}	0.72 ± 0.01^{e}	7.5 ± 0.2^{ijk}	8.2 ± 0.2^{hij}
21			0.37 ± 0.01^{u}	0.06 ± 0.01^{m}	0.44 ± 0.01^{s}	0.40 ± 0.04^{klm}	7.8 ± 0.6^{hi}	8.2 ± 0.7^{hi}
22			0.75 ± 0.01^{n}	0.10 ± 0.01^{hij}	0.86 ± 0.01^{l}	0.51 ± 0.02^{hi}	7.6 ± 0.5^{hijk}	8.1 ± 0.6^{hijk}
23			0.68 ± 0.01^{op}	0.12 ± 0.01^{fg}	0.80 ± 0.01^{m}	0.34 ± 0.01^{nop}	7.52 ± 0.03^{hijk}	7.87 ± 0.02^{ijkl}
24		Cambisol	1.07 ± 0.02^{j}	0.11 ± 0.01^{gh}	1.18 ± 0.02^{j}	0.33 ± 0.04^{op}	7.2 ± 0.2^{k}	7.6 ± 0.2^{l}
25			0.66 ± 0.01^{p}	0.09 ± 0.01^{ijk}	0.76 ± 0.01^{n}	0.31 ± 0.02^{pq}	5.4 ± 0.1^{n}	5.7 ± 0.1^{o}
26			0.39 ± 0.03^{u}	0.06 ± 0.01^{m}	0.46 ± 0.04^{rs}	0.64 ± 0.11^{fg}	7.76 ± 0.09^{hij}	8.4 ± 0.2^{gh}
27			0.71 ± 0.01^{o}	0.14 ± 0.04^{de}	0.85 ± 0.04^{l}	0.61 ± 0.04^{fg}	8.94 ± 0.08^{e}	9.6 ± 0.1^{c}
28			0.55 ± 0.02^{q}	0.11 ± 0.01^{gh}	0.66 ± 0.01^{o}	0.44 ± 0.01^{jk}	9.6 ± 0.4^{c}	10.1 ± 0.4^{b}
29			0.52 ± 0.02^{qr}	0.10 ± 0.01^{hij}	0.62 ± 0.03^{op}	0.37 ± 0.01^{mno}	7.28 ± 0.05^{k}	7.65 ± 0.06^{l}
30			0.91 ± 0.03^{l}	0.13 ± 0.01^{ef}	1.04 ± 0.01^{k}	0.33 ± 0.01^{nop}	7.76 ± 0.08^{hij}	8.09 ± 0.08^{hijk}
31			0.26 ± 0.01^{x}	0.07 ± 0.01^{lm}	0.33 ± 0.01^{t}	0.41 ± 0.01^{klm}	7.3 ± 0.6^{k}	7.8 ± 0.6^{kl}
32			1.41 ± 0.05^{f}	0.18 ± 0.01^{b}	1.59 ± 0.05^{f}	0.38 ± 0.02^{lmn}	6.6 ± 0.2^{l}	7.0 ± 0.1^{m}
33			0.78 ± 0.01^{n}	0.11 ± 0.01^{ghi}	0.88 ± 0.01^{l}	0.41 ± 0.41^{klm}	8.8 ± 0.3^{ef}	9.2 ± 0.3^{cd}
34			0.50 ± 0.03^{r}	0.13 ± 0.01^{ef}	0.63 ± 0.04^{op}	0.40 ± 0.01^{klm}	6.7 ± 0.4^{l}	7.1 ± 0.4^{m}

tr – traces; Different letters in the same column show no significant difference between means according to Tukey's HSD test ($p > 0.05$).

3.4. Fatty acids

The fatty acid composition of the Armuña lentils studied was also evaluated and the results are presented in Table 5. A total of 19 different compounds were identified (Table S1), with C18:2n6c (linoleic acid, 26.3 to 47.1 %), C18:1n9c (oleic acid, 20.9 to 34.1 %), C18:3n3 (α-linolenic, 9.6 to 15.1 %), and C16:0 (palmitic acid, 10.6 to 14.53 %) standing out as the most prevalent fatty acids in all of the samples analyzed. Furthermore, as to their classification it is worth stressing that polyunsaturated fatty acids (PUFAs) are those present in higher proportions (45.3 to 63.7 %) followed by monounsaturated (20.9 to 34.9 %), and saturated (15.4 to 21.6 %) fatty acids (MUFAs and SFAs respectively). These features reflect an excellent fatty acid profile and substantiate the inclusion of this type of lentils in a balanced diet. The fact that linoleic and α-linolenic acids are precursors of omega-6 and omega-3 fatty acids, which are not synthesized by the human organism, strengthens the idea that the regular consumption of lentils is associated with several beneficial effects on human health and wellbeing (Pereira, Barros, & Ferreira, 2015). The results obtained coincide with those of our previous study on Pardina lentils (Liberal et al., 2021) in which similar amounts of the most prevalent fatty acids were identified. In addition, in the aforementioned study PUFAs are in first place with values ranging from 53.1 to 60.95 %, followed by SFAs (20.54–27.16 %) and MUFAs (15.46–27.16 %). Similar fatty acid profiles were identified in different lentil varieties of various origins, which may indicate a weak correlation of this parameter with different cultivars and climate conditions (Zhang et al., 2014; Zia-Ul-Haq et al., 2011). The analysis of variance showed a negligible correlation between the concentration of fatty acids and the growing season and soil type (table 2).

3.5. Antioxidant activity evaluation

There are several methods for evaluating the in vitro antioxidant activity of biologically active substances, ranging from chemical assays with lipid substrates to more complex assays using the most diverse instrumental techniques. Due to the different types of free radicals and their different ways of acting in living organisms, there will hardly be a simple and universal method by which antioxidant activity can be measured precisely and quantitatively (Sánchez-Moreno, 2002). In this study therefore, two in vitro methods of assessing antioxidant activity were used, namely the TBARS assay and the OxHLIA assay.

The antioxidant activity of the decocted extracts of Armuña lentils was assessed by examining their capacity to inhibit lipid peroxidation and oxidative hemolysis using two in vitro assays, namely TBARS and OxHLIA. The results obtained are presented in Table 6. In the TBARS assay decocted extracts were found to show high antioxidant activity, with EC_{50} values ranging from 30 to 99 μg/mL. As for the OxHLIA assay, Armuña lentils demonstrated their ability to prevent 50 % of the erythrocyte population from oxidizing; this is triggered by AAPH (2.20-azobis/2-methylpropionamidine) dihydrochloride; the oxidizing agent) for 60 min, with IC_{50} values fluctuating between 67 and 673 μg/mL. Despite the good results obtained, in both assays the values were higher than those of the positive Trolox control, showing no significant differences between samples ($p > 0.05$). Among legumes, the Trolox equivalent antioxidant capacities of lentils have been shown to be significantly higher than those of other legumes (Han & Baik, 2008). However, this capacity differs according to the lentil varieties studied as a lower antioxidant capacity was observed in Pardina lentils (Liberal et al., 2021), in which higher concentrations of decocted extracts were necessary to inhibit lipid peroxidation (211 to 228 μg/mL) and oxidative

Table 5
Fatty acid composition of the Armuña lentils studied (%) (mean ± SD).

Samples	Year	Soil type	C16:0	C18:0	C18:1n9c	C18:2n6c	C18:3n3	SFA	MUFA	PUFA
1	2018	Luvisol	12.4 ± 0.1	3.18 ± 0.04	24.4 ± 0.1	35.8 ± 0.1	15.1 ± 0.2	20.8 ± 0.1^{c}	25.4 ± 0.1^{mn}	53.8 ± 0.1^{l}
2			11.8 ± 0.2	2.64 ± 0.02	24.4 ± 0.4	40.4 ± 0.2	13.9 ± 0.1	17.8 ± 0.2^{k}	25.4 ± 0.4^{mn}	56.8 ± 0.2^{f}
3			11.8 ± 0.2	2.64 ± 0.02	24.03 ± 0.08	40.4 ± 0.2	14.3 ± 0.6	17.8 ± 0.2^{k}	25.0 ± 0.1^{opq}	57.1 ± 0.3^{ef}
4			13.2 ± 0.4	2.7 ± 0.1	24.0 ± 0.1	38.6 ± 0.5	14.2 ± 0.2	19.1 ± 0.4^{fg}	25.4 ± 0.1^{mno}	55.5 ± 0.5^{gh}
5			13.9 ± 0.3	3.2 ± 0.1	26.0 ± 0.3	36.22 ± 0.08	13.2 ± 0.1	20.7 ± 0.4^{c}	27.1 ± 0.4^{k}	52.2 ± 0.1^{n}
6			11.3 ± 0.01	0.21 ± 0.01	26.3 ± 0.1	39.0 ± 0.2	14.06 ± 0.05	16.9 ± 0.2^{lm}	27.6 ± 0.1^{ij}	55.5 ± 0.2^{gh}
7			0.13 ± 0.01	2.8 ± 0.7	22.65 ± 0.09	38.4 ± 0.5	12.6 ± 0.2	21.6 ± 0.6^{b}	23.8 ± 0.2^{r}	54.6 ± 0.4^{jk}
8			11.1 ± 0.3	2.78 ± 0.06	27.09 ± 0.2	39.38 ± 0.05	13.5 ± 0.4	16.8 ± 0.2^{lmn}	28.3 ± 0.2^{fg}	54.9 ± 0.4^{ij}
9			13.2 ± 0.4	2.7 ± 0.1	24.0 ± 0.1	38.6 ± 0.5	14.2 ± 0.2	19.1 ± 0.4^{fg}	25.4 ± 0.1^{mno}	55.5 ± 0.5^{gh}
10		Cambisol	12.4 ± 0.3	3.36 ± 0.05	27.7 ± 0.7	35.6 ± 0.8	12.7 ± 0.3	19.7 ± 0.3^{e}	29.2 ± 0.6^{e}	51 ± 1^{o}
11			11.5 ± 0.5	2.8 ± 0.1	25.3 ± 0.2	39.2 ± 0.1	13.4 ± 0.6	17.8 ± 0.6^{k}	26.7 ± 0.2^{l}	55.5 ± 0.4^{gh}
12			11.3 ± 0.3	2.9 ± 0.1	26.3 ± 0.1	39.0 ± 0.2	14.06 ± 0.05	16.9 ± 0.2^{lm}	27.6 ± 0.1^{ij}	55.5 ± 0.2^{gh}
13			11.7 ± 0.5	3.07 ± 0.01	25.9 ± 0.3	39.3 ± 0.2	13.5 ± 0.6	18.0 ± 0.5^{k}	27.4 ± 0.3^{jk}	55.4 ± 0.8^{hi}
14			12.81 ± 0.04	3.2 ± 0.1	26.2 ± 0.1	38.33 ± 0.06	13.01 ± 0.2	18.9 ± 0.1^{gh}	27.4 ± 0.1^{jk}	53.7 ± 0.2^{l}
15			12.7 ± 0.2	3.1 ± 0.1	26.8 ± 0.4	38.7 ± 0.4	12.9 ± 0.4	18.6 ± 0.2^{ij}	27.8 ± 0.3^{hi}	53.6 ± 0.1^{l}
16		Not a PGI	10.9 ± 0.2	2.65 ± 0.01	23.5 ± 0.7	43.3 ± 0.3	12.4 ± 0.4	16.9 ± 0.1^{lm}	24.7 ± 0.7^{q}	58.3 ± 0.7^{d}
17			11.29 ± 0.05	2.60 ± 0.05	24.7 ± 0.9	40.8 ± 0.3	14.2 ± 0.2	16.8 ± 0.1^{mn}	25.7 ± 0.1^{m}	57.6 ± 0.1^{e}
18	2019	Luvisol	13.7 ± 0.4	3.85 ± 0.06	32.5 ± 0.2	34.5 ± 0.3	10.34 ± 0.08	20.1 ± 0.3^{d}	33.3 ± 0.2^{cd}	46.6 ± 0.6^{p}
19			11.4 ± 0.8	2.6 ± 0.1	22.9 ± 0.2	42.8 ± 0.2	13.8 ± 0.3	17.1 ± 0.8^{l}	24.0 ± 0.2^{r}	58.8 ± 0.6^{c}
20			13.3 ± 0.2	3.84 ± 0.03	34.1 ± 0.1	34.7 ± 0.1	9.6 ± 0.1	19.5 ± 0.2^{ef}	34.9 ± 0.1^{a}	45.7 ± 0.1^{qr}
21			12.76 ± 0.1	3.24 ± 0.04	27.6 ± 0.2	39.1 ± 0.1	11.8 ± 0.3	18.7 ± 0.1^{hij}	28.5 ± 0.2^{f}	52.8 ± 0.2^{m}
22			12.7 ± 0.1	3.24 ± 0.01	27.61 ± 0.04	39.1 ± 0.2	11.8 ± 0.1	18.7 ± 0.1^{hij}	28.5 ± 0.2^{f}	52.8 ± 0.2^{m}
23			13.7 ± 0.2	3.2 ± 0.1	22.6 ± 0.1	41.9 ± 0.4	10.78 ± 0.02	20.1 ± 0.3^{d}	22.8 ± 0.1^{s}	57.1 ± 0.2^{f}
24		Cambisol	12.23 ± 0.05	3.02 ± 0.05	24.2 ± 0.1	41.3 ± 0.1	12.3 ± 0.3	18.8 ± 0.1^{ghi}	25.3 ± 0.1^{nop}	55.9 ± 0.2^{g}
25			13.5 ± 0.2	3.5 ± 0.1	26.97 ± 0.01	37.8 ± 0.3	11.43 ± 0.09	20.7 ± 0.2^{c}	28.0 ± 0.1^{gh}	51.3 ± 0.2^{o}
26			12.9 ± 0.4	3.5 ± 0.1	23.1 ± 0.3	40.2 ± 0.4	11.9 ± 0.1	19.7 ± 0.5^{de}	25.0 ± 0.3^{pq}	55.2 ± 0.2^{hi}
27			14.53 ± 0.01	4.0 ± 0.3	33.1 ± 0.5	34.1 ± 0.1	9.8 ± 0.3	20.8 ± 0.3^{c}	33.9 ± 0.6^{b}	45.3 ± 0.2^{r}
28			14.53 ± 0.01	4.1 ± 0.3	33.1 ± 0.5	34.1 ± 0.1	9.9 ± 0.3	20.8 ± 0.3^{c}	33.9 ± 0.6^{b}	45.3 ± 0.2^{r}
29			12.8 ± 0.1	3.24 ± 0.04	27.6 ± 0.2	39.1 ± 0.1	11.8 ± 0.3	18.7 ± 0.1^{hij}	28.5 ± 0.2^{f}	52.8 ± 0.2^{m}
30			11.3 ± 0.3	2.4 ± 0.1	22.1 ± 0.1	44.9 ± 0.4	13.5 ± 0.1	16.5 ± 0.5^{n}	23.0 ± 0.1^{s}	60.6 ± 0.5^{b}
31			13.72 ± 0.09	4.12 ± 0.01	32.7 ± 0.1	34.1 ± 0.2	10.3 ± 0.2	20.6 ± 0.1^{c}	33.5 ± 0.1^{c}	45.9 ± 0.1^{q}
32			12.2 ± 0.1	3.15 ± 0.06	26.3 ± 0.2	39.7 ± 0.1	13.0 ± 0.3	18.4 ± 0.2^{j}	27.2 ± 0.2^{k}	54.4 ± 0.4^{k}
33			12.6 ± 0.4	3.5 ± 0.2	25.3 ± 0.1	39.5 ± 0.6	11.7 ± 0.2	19.7 ± 0.6^{e}	25.4 ± 0.1^{mn}	54.9 ± 0.6^{ij}
34			10.6 ± 0.2	2.19 ± 0.07	20.9 ± 0.6	47.1 ± 0.8	12.7 ± 0.2	15.4 ± 0.3^{o}	20.9 ± 0.6^{f}	63.7 ± 0.9^{a}

C16:0 - palmitic acid; C18:0 – stearic acid; C18:1n9 - oleic acid; C18:2n6 - linoleic acid; C18:3n3 - α-linolenic acid; SFA- Saturated fatty acids; MUFA- Monounsaturated fatty acids; PUFA- Polyunsaturated fatty acids. Different letters in the same column show no significant difference between means according to Tukey's HSD test ($p > 0.05$).

Table 6
Antioxidant activity of the Armuña lentils studied (mean ± SD).

Samples	Year	Type of soil	OxHLIA (IC_{50}; μg/mL); Δt = 60 min	TBARS (EC_{50}. μg/mL)
1	2018	Luvisol	81 ± 3	35 ± 3^{p}
2			121 ± 5	45 ± 4^{o}
3			120 ± 5	55 ± 3^{klm}
4			67 ± 3	59 ± 1^{ijkl}
5			99 ± 3	32 ± 9^{p}
6			97 ± 3	35 ± 9^{p}
7			162 ± 5	63 ± 3^{hij}
8			90 ± 3	85 ± 2^{cd}
9			125 ± 3	86 ± 2^{bcd}
10		Cambisol	120 ± 4	58 ± 2^{ijkl}
11			206 ± 5	54 ± 4^{lmn}
12			100 ± 4	62 ± 1^{hijk}
13			111 ± 4	59 ± 1^{ijkl}
14			184 ± 6	47 ± 1^{mno}
15			91 ± 3	46 ± 2^{mno}
16		No PGI	109 ± 4	40 ± 3^{op}
17			198 ± 5	30 ± 2^{g}
18	2019	Luvisol	430 ± 6	72 ± 11^{ef}
19			171 ± 4	61 ± 9^{fgh}
20			243 ± 10	72 ± 3^{de}
21			203 ± 10	86 ± 1^{bcd}
22			426 ± 24	91 ± 3^{bcd}
23			123 ± 5	65 ± 5^{ghi}
24		Cambisol	300 ± 8	82 ± 7^{ijkl}
25			289 ± 7	81 ± 4^{fg}
26			175 ± 12	47 ± 26^{de}
27			118 ± 6	56 ± 18^{mno}
28			183 ± 17	86 ± 3^{jkl}
29			534 ± 17	93 ± 2^{bc}
30			673 ± 16	99 ± 10^{b}
31			94 ± 3	70 ± 11^{no}
32			188 ± 6	83 ± 17^{fg}
33			183 ± 5	63 ± 53^{cd}
34			397 ± 7	76 ± 6^{a}

EC_{50}: extract concentration corresponding to 50 % of antioxidant activity. Trolox EC_{50} values: 23 μg/mL (TBARS inhibition) and 21.8 μg/mL (OxHLIA Δt = 60 min); Different letters in the same column show significant difference between means according to Tukey's HSD test ($p < 0.05$).

hemolysis (178 μg/mL to 1312 μg/mL, Δ120 min). The results presented combine to substantiate the potential of Armuña lentils as natural antioxidants which can be used in different formulations.

Taking into account our results from studying Armuña lentils, antioxidant activity showed a strong correlation with the season of cultivation of the lentils (Table 2). TBARS and OxHLIA were the most highly correlated of all the parameters analyzed in this study. This result could be explained by the fact that the bioactive compounds responsible for antioxidant activity in lentils are generally secondary metabolites such as phenolic compounds (Oomah, Caspar, Malcolmson, & Bellido, 2011) and tocopherols (Fernández-Orozco, Zielinski, & Piskuła, 2003). This implies that the amount of these compounds increases when the plant undergoes a stressful situation such as the temperatures to which it is subjected, the amount of water it receives, and the amount of radiation owing to the presence of pathogens and/or insects (Jaballah et al., 2017). Climatic conditions in different seasons can therefore strongly affect the antioxidant activity of lentils.

4. Conclusions

Armuña lentils were analyzed to examine their nutritional and chemical composition and also their antioxidant capacity. Our research found large amounts of valuable nutrients such as proteins, fibers, and minerals in addition to interesting amounts of organic acids and tocopherols. As is characteristic of this type of pulse, low concentrations of fat and sugars were detected in the samples analyzed, which reinforces the potential of its consumption as part of low-fat and low-glycemic diets. Likewise, their high protein content makes Armuña lentils a good meat substitute to be used in vegetarian diets. No significant differences were found between the various lentil cultivars regarding the nutritional, chemical and antioxidant characteristics.

It was also demonstrated that the nutritional and chemical profiles of the Armuña lentil are significantly influenced by the year of cultivation and consequently by the climatic changes which occur during the growing season. These have been shown to have a significant effect on the major nutrients in lentils, such as the concentration of fat, ashes, fibers, and sugars, namely fructose. To a lesser extent, proteins and sucrose were also influenced by this parameter. Conversely, the climatic conditions appear not to affect the moisture content, the total number of carbohydrates, the energy, and the concentrations of organic acids and fatty acids. Also, it was also possible to conclude that the different soil types in which the lentils studied grew did not significantly affect any of the parameters analyzed, which may be related to their specific and individual composition.

The results obtained show that the selection of lentil varieties with higher amounts of nutritional compounds must take into account not only genetic factors but also environmental parameters and dominant edaphic characteristics during cultivation, as the quality characteristics which stand out in this type of pulse appears to be influenced by these factors. In the future, the knowledge of which characteristics are mostly influenced, not only by environmental factors but also by the composition of different soil types, should be further investigated. This information should be preponderant in improving the nutritional and chemical composition of lentils, from which new food products rich in protein, fiber, and minerals, among other valuable compounds, can be formulated.

CRediT authorship contribution statement

Ângela Liberal: Methodology, Software, Validation, Investigation, Data curation, Writing – original draft. **Daiana Almeida:** Methodology, Data curation. **Ângela Fernandes:** Conceptualization, Validation, Investigation, Data curation, Writing – review & editing, Supervision. **Carla Pereira:** Methodology, Data curation. **Isabel C.F.R. Ferreira:** Project administration. **Ana María Vivar-Quintana:** Conceptualization, Visualization, Supervision, Writing – review & editing. **Lillian Barros:** Validation, Investigation, Writing – review & editing, Visualization, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgments

The authors are grateful to the Foundation for Science and Technology (FCT, Portugal) for financial support from the FCT/MCTES (PIDDAC) national funds to CIMO (UIDB/00690/2020 and UIDP/00690/2020) and SusTEC (LA/P/0007/2021), and also for the national funding by FCT and P.I. in the form of the institutional scientific employment program for the contracts of L. Barros, Â. Fernandes, and C. Pereira, and the PhD fellowship (2021.04585.BD) of Â. Liberal. A.M. Vivar-Quintana is also grateful for the support of the University of Salamanca regarding researcher mobility and would like to thank the Regulatory Council of the "Lenteja de la Armuña" PGI for the lentil samples.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2023.135491.

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