Research Article

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Nutritional, chemical, and antioxidant screening of selected varieties of lentils (*Lens culinaris* spp.) from organic and conventional agriculture

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Abstract

BACKGROUND: Lentils are an ancient legume established worldwide for direct consumption and with great potential for application in food processing. In addition, it is a sustainable crop owing to its ability to scavenge nitrogen and carbon, and it improves the nutrient status of the soil. A diet rich in lentils has been linked to significant health benefits. However, the composition of lentils can be influenced by both the lentil variety and the growing conditions. The aim of this work was to evaluate the nutritional profiles and antioxidant potential, as well as the impact that the type of cultivation (conventional or organic) and the variety could have on these parameters, in different lentil varieties.

RESULTS: Overall, carbohydrates are the major macronutrients in all varieties, with notable amounts of fibre (11.62–27.36%) and starch (41.98–50.27%). High amounts of protein and ash were also identified, particularly in the Beluga variety, with 21.9–23.3 and 1.38–1.82 g 100 g^{-1} fresh weight, respectively. Fructose and sucrose were detected (high-performance liquid chromatography (HPLC) with refraction index detection), along with oxalic, quinic, malic, and shikimic acids (ultra-fast liquid chromatography with photodiode array detection), and α- and γ-tocopherol isoforms (HPLC with fluorescence detection). Fatty acid methyl ester assessment showed the prevalence of polyunsaturated fatty acids (33.5–46.3%). Good antioxidant capacity (thiobarbituric acid reactive substances and oxidative haemolysis inhibition assay) was also noted.

CONCLUSION: The results obtained showed that all the varieties analysed are an excellent source of fibre and have a good antioxidant capacity. Lentil variety has a greater influence on its nutritional composition than the type of cultivation.

Keywords: antioxidant activity; *Lens culinaris*; nutritional and chemical composition; organic and conventional farming

INTRODUCTION

In recent decades, interest in nutrition research has grown exponentially and is now recognised as a critical factor: on the one hand, because of its importance from a dietary point of view; and on the other because of its contribution to prevent and/or stop the development of several acute and chronic diseases, mainly those triggered by insufficient, excessive, or unbalanced nutrient intake.[1] Consequently, in modern society, changes in dietary habits have promoted the search for new sources of macro- and micronutrients. The challenge is to find foods able to meet the nutritional needs of different population groups, both for human and animal consumption.[2]

Leguminous seeds, also known as pulses, are a worldwide staple food belonging to the Leguminosae (or Fabaceae) family, composed of more than 13 000 distinct species. These represent one of the most economically important crops worldwide, just below cereals, providing about 33% of protein, carbohydrates, fibre,

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minerals, and vitamins, among other phytochemicals, hence being acknowledged as an excellent source of nutrients.[3,4] Lentils (*Lens culinaris*) are an ancient pulse that embodies one of the oldest food crops cultivated globally. This type of pulse holds about twice the amount of protein than whole grains such as wheat, oats, barley, and rice, being responsible for about a third of its total energetic value.[5,6] Worldwide, about 6.10 million ha are allocated to lentil cultivation, with an annual production and yield of 6.33 million tons and 1038 kg ha^{-1}, respectively.[7] In recent years, world production of lentils has increased, with countries in North America and Asia accounting for most of the production of this pulse.[8] Also, in some areas such as West Asia and the Indian subcontinent, lentils epitomise the cheapest source of protein, meeting the nutritional needs of a largely vegetarian population. They are sources of essential amino acids such as leucine, lysine, threonine, and phenylalanine, yet lacking in methionine and cysteine.[9] The combination of lentils with cereals such as rice or wheat, however, establishes an effective strategy for the intake of all essential amino acids, including those that are not present in these pulses' composition.[5] Lentils are also known as rich sources of carbohydrates, such as fibre, made partly by prebiotics, and starch, which may decrease the glycaemic index of foods.[10-12] Additionally, vitamin B complex vitamins, especially folate (B9), and minerals such as iron, deficiency of which is widespread throughout the world, seem to occur in notable amounts in these types of pulse.[5] Simultaneously, lentils are made up of proteins and peptide fractions able to modulate the inhibitory activity of angiotensin-converting enzyme after ingestion, thus reducing the risk of hypertension.[13] Given the valuable nutritional and chemical profile of lentils, their inclusion in a daily diet can establish a healthy lifestyle, avoiding diseases that are increasingly common in society, such as cancer, diabetes, cardiovascular diseases, and other acute and chronic conditions.[5] Lentils are known to occur in distinct colours, with the outer shell varying from light green to dark purple, mottled, grey, brown, or black. Additionally, they may differ with regard to cultivar, composition of the seed coat and cotyledons, which, in turn, can be red, yellow, or green.[9] The main classes of lentils available on the market are the green and red types, the former being sold as whole seed and the latter as whole seed, husked and/or divided.[5,14] Several popular varieties of lentils include Spanish brown (Pardina), French green (Puy, dark speckled blue-green), Red chief, Beluga, Eston, and Milestone (small green), Grandora (large green), Masoor (brown-skinned lentils that are orange inside), Petite Golden, and Crimson/red (decorticated masoor lentils), among others.[9,15]

Since the beginning of the 21st century, the marketing of organic products has grown exponentially worldwide. There is a growing consumer perception of the quality and environmental impact of the consumption and production of organic products.[16,17] However, there still seems to be a large gap in the scientific evidence that some are effectively different and/or better than those conventionally produced in terms of product, nutrition, and health criteria.[18,19] On the other hand, the results are highly dependent on how the analysis is carried out. However, some studies show that plant products from organic farming contain fewer pesticide residues and other unique compounds when compared to their counterparts from conventional farming, these differences being of statistical significance. However, it is not clear to what extent they can affect human health.[20,21]

In this study we therefore aim to investigate the nutritional, chemical, and antioxidant composition of some of the most common lentil varieties, namely Beluga, Red, Castellana, and Stone, inferring whether these variables are somehow affected by the type of cultivation (conventional and organic farming), and by the variety of the lentil seeds themselves, acknowledging the different lentil varieties and their possible benefits for human health and nutrition, concurrently investigating possible influence of the type of agriculture used in their cultivation.

MATERIALS AND METHODS

Samples

Four lentil varieties, namely Beluga, Castellana, Stone, and Red – some of the most widely consumed varieties around the world – were purchased in different Spanish markets in two different cities (Salamanca and Zamora), originating from different countries (USA, Canada, Spain, and Australia), as described in Table 1. For each variety, one of the samples was organically farmed and the other two conventionally farmed. All samples were collected in the 2019 season and all analyses were performed before 2 years of storage. The outer skin was black, brown, mottled-green, and red, respectively, covering the whole grain. The grains of the red variety were analysed without husk. The organic lentils purchased were labelled with the European Union seal guaranteeing certification of organic production. The samples were cleaned to remove foreign substances and injured grains, before grinding in a Knifetec

Table 1. Sample characterization according to variety, country of origin and type of production

Variety	Sample	Country of origin	Type of production
Beluga	BE01	Spain	Conventional agriculture
	BA01	Australia	Conventional agriculture
	BU01E	USA	Organic agriculture
Red	RC01	Canada	Conventional agriculture
	RC02	Canada	Conventional agriculture
	RC01E	Canada	Organic agriculture
Castellana	CE01	Spain	Conventional agriculture
	CE02	Spain	Conventional agriculture
	CE01E	Spain	Organic agriculture
Stone	SC01	Canada	Conventional agriculture
	SC02	Canada	Conventional agriculture
	SE01E	Spain	Organic agriculture

1095 mill (FOSS, Hilleroed, Denmark) at a monitored temperature (20 °C) and stored pending additional laboratory analysis.

Nutritional and energetic value

The nutritional profile of the samples under investigation, namely protein, carbohydrates, and ash content, was assessed by applying AOAC methodologies.[22] In short, crude protein was ascertained using the macro-Kjeldahl procedure (N × 6.25), crude fat was estimated through the extraction of a known weight of each sample with petroleum ether in a Soxhlet device, and ash by incinerating samples at 550 ± 10 °C. Total carbohydrates were assessed by difference [100 − (g proteins + g fat + g ash)], and energetic value according to the equation: energy value [kcal 100 g^{-1} fresh weight (fw)] = 4 × (g protein + g carbohydrates) + 9 × (g fat). Moisture content was assessed by difference, weighing samples immediately after harvest and again after oven-drying at 105 °C.

An enzymatic–gravimetric method, using enzymatic digestion with α-amylase, protease, and amyloglucosidase, was employed to assess the total dietary fibre content according to the earlier cited methods. Briefly, dried lentil samples were incubated with α-amylase at ∼100 °C, promoting the gelatinization, hydrolysis, and depolymerisation of the starch, followed by further incubations at 60 °C with protease (to solubilise and depolymerise proteins) and amyloglucosidase (to hydrolyse starch fragments to glucose). Therefore, samples were treated with four volumes of ethanol to precipitate soluble fibre and remove depolymerised protein and glucose. The residues were then filtered, washed with 78% and 95% ethanol and acetone, dried, and weighed. One duplicate was analysed for protein and the other was incubated at 525 °C to determine ash content. All experiments were analysed in duplicate, and the results are given in relative percentage following the equation: % total dietary fibre = [(*R* − *P* − *A*)/SW] × 100, where *R* is the average residue, *P* is the average protein, *A* is the average ash and SW is the average weight of the samples. Starch content was established as defined by Alajaji and El-Adawy,[23] as reducing sugars after complete acid hydrolysis.

Chemical composition

Free sugars

The sugar profile was determined after an extraction procedure, as described by Spréa *et al.*,[24] by high-performance liquid chromatography (HPLC) coupled to a refraction index detector. Melezitose was used as internal standard (IS). Subsequent identification and quantification of each spotted compound were accomplished by comparison of the retention times from authentic standards and using the IS method and calibration curves of each one of them, respectively. Free sugar concentrations are given in grams 100 g^{-1} fw.

Organic acids

Organic acids analysis was performed by ultra-fast liquid chromatography coupled with a photodiode array detector (Shimadzu Corporation, Kyoto, Japan), using 215 nm as preferred wavelength, and following a methodology previously described and optimized by Pereira *et al.*[25] In brief, all samples (∼1.5 g) were obtained by stirring with 25 mL metaphosphoric acid for 25 min, and then filtering through Whatman No. 4 paper. Quantification of each spotted compound was accomplished by comparing the peak area with calibration curves from commercial standards of each compound, while identification was completed by comparing the obtained chromatograms of the analysed samples with those of commercial standards. Results are given in grams 100 g^{-1} fw.

Tocopherols

Tocopherol analyses were achieved employing an HPLC system, and a fluorescence detector (FP-2020, JASCO, Easton, MD, USA) programmed for excitation at 290 nm and emission at 330 nm, after a procedure described earlier.[24] Separation of the tocopherol isoforms was achieved using HPLC equipment operating under the following conditions: system: pump (Smartline system 1000, Knauer, Berlin, Germany), degasser system (Smartline manager 5000), autosampler (JASCO AS-2057); column: Polyamide II (250 mm × 4.6 mm i.d.) normal-phase column from YMC Waters (Dinslaken, Germany); detector: fluorescence detector (JASCO FP-2020); mobile phase: hexane and ethyl acetate (70:30, v/v); flux: 1 mL min^{-1}; wavelength: excitation at 290 nm and emission at 330 nm; temperature: 35 °C; software: DataApex Clarity, Version 2.4.1.43. Identification was performed via chromatographic comparison with authentic standards and quantification established on the fluorescence signal reaction of each standard, using the IS (tocol) method and calibration curves from commercial standards of each compound. The results are given in milligrams 100 g^{-1} fw.

Fatty acids

The samples were subjected to a transesterification process of the lipid fraction from Soxhlet extraction,[24] and fatty acid methyl esters (FAME) determined by gas–liquid chromatography with flame ionization detection, utilizing a YOUNG IN Chomass 6500 GC system instrument (Gyeonggi, South Korea) supplied with a split/splitless injector set at 250 °C with a split ratio of 1:50, a flame ionization detector set at 260 °C, and a Zebron-Fame column (30 m × 0.25 mm i.d. × 0.20 µm df; Phenomenex, Lisbon, Portugal). The following oven temperature program was used: initial temperature 100 °C, held for 2 min, increase 10 °C min^{-1} to 140 °C, followed by a 3 °C min^{-1} ramp to 190 °C and 30 °C min^{-1} ramp to 260 °C. The carrier gas (hydrogen) flow rate was 1.2 mL min^{-1}, measured at 250 °C. Fatty acid identification and quantification were performed by comparing the relative retention times of FAME peaks from samples with standards (standard mixture 47 885-U, Sigma, St Louis, MO, USA) and results were recorded and processed using Clarity DataApex 4.0 software (Prague, Czech Republic) and expressed as a relative percentage of each fatty acid. Identification and quantification of each FAME was accomplished by comparison of the relative retention times of the samples with commercial standards (standard mixture 47 885-U, Sigma). The results were documented and treated using Clarity DataApex 4.0 software and stated as a relative percentage of each fatty acid.

Antioxidant activity evaluation

Extract preparation

Dry sample materials were used to prepare the decocted extracts, combining 100 mL distilled water with ∼3 g of each sample. The mixture was boiled for 10 min and then filtrated through Whatman No. 4 paper. The extracts were then frozen and lyophilized (FreeZone 4.5, Labconco, Kansas City, MO, USA) into a dried extract and stored at room temperature until further analysis.

Thiobarbituric acid reactive substances (TBARS)

The lyophilized decoction extracts were used for TBARS assay, these being dissolved in distilled water and diluted from 0.3125 to 0.01953 mg mL^{-1}. The decrease in TBARS was evaluated by

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Table 2. Nutritional value (g 100 g^{-1} fw) and energetic value (kcal 100 g^{-1} fw) of the studied Beluga, Red, Castellana, and Stone lentils ($n = 3$; mean ± SD)

Sample	Total carbohydrates	Starch (%)	Fiber (%)	Proteins	Ash	Fat	Moisture (%)	Energy value
Beluga								
BA01	74.4 ± 0.6e	46.3 ± 0.2b	24.91 ± 0.01b	23.3 ± 0.7a	1.4 ± 0.4cde	0.8 ± 0.6cde	8.99 ± 0.01bc	399 ± 3bc
BE01	74.5 ± 0.4e	42.6 ± 0.2e	27.36 ± 0.01a	22.9 ± 0.3a	1.38 ± 0.04def	1.2 ± 0.2a	9.30 ± 0.01de	491.1 ± 0.7a
BU01E	75.7 ± 0.5d	41.98 ± 0.02f	23.13 ± 0.01c	21.9 ± 0.9c	1.82 ± 0.09a	0.7 ± 0.2ef	9.88 ± 0.01f	486.9 ± 0.3e
Red								
RC01	76.5 ± 0.2c	50.1 ± 0.1a	11.62 ± 0.01k	21.3 ± 0.5d	1.4 ± 0.1def	0.80 ± 0.07de	8.83 ± 0.01b	583.9 ± 0.1bc
RC02	75.3 ± 0.3d	50.3 ± 0.2a	12.37 ± 0.01j	22.3 ± 0.6bc	1.3 ± 0.2ef	1.05 ± 0.01ab	9.29 ± 0.01de	490.4 ± 0.6ab
RC01E	76.50 ± 0.08c	45.8 ± 0.1c	13.87 ± 0.01i	20.91 ± 0.08de	1.57 ± 0.03bc	1.0 ± 0.2abc	8.81 ± 0.01b	586.7 ± 0.5bc
Castellana								
CE01	74.7 ± 0.2e	42.1 ± 0.1d	16.22 ± 0.01h	23.1 ± 0.6a	1.7 ± 0.3ab	0.56 ± 0.04f	8.49 ± 0.01a	488.1 ± 0.6e
CE02	76.29 ± 0.04c	45.44 ± 0.09a	15.07 ± 0.01h	21.1 ± 0.1d	1.78 ± 0.07a	0.8 ± 0.1de	8.32 ± 0.01a	582.3 ± 0.2de
CE01E	75.4 ± 0.5d	41.54 ± 0.06b	17.69 ± 0.01f	22.4 ± 0.6b	1.3 ± 0.1f	0.95 ± 0.17bcd	9.42 ± 0.01e	491.4 ± 0.9ab
Stone								
SC01	77.7 ± 0.3b	45.3 ± 0.2d	19.59 ± 0.01d	20.4 ± 0.6ef	1.5 ± 0.2bcd	0.74 ± 0.03ef	9.32 ± 0.01e	397.8 ± 0.3cd
SC02	77 ± 1a	46.7 ± 0.3a	16.84 ± 0.01g	20.0 ± 0.1f	1.47 ± 0.09cde	0.76 ± 0.01de	9.25 ± 0.01de	398.4 ± 0.8cd
SE01E	74.81 ± 0.01d	48.1 ± 0.2b	17.79 ± 0.01e	22.3 ± 0.1bc	1.3 ± 0.1ef	0.75 ± 0.01e	9.13 ± 0.01cd	398.3 ± 0.3bc

Note: Different letters in the same column indicate significant differences between means according to Tukey's HSD test ($P < 0.05$).

Table 3. Composition of sugars (g 100 g^{-1} fw) of the studied Beluga, Red, Castellana, and Stone lentils ($n = 3$; mean ± SD)

Sample	Fructose	Sucrose	Total sugars
Beluga			
BA01	1.64 ± 0.01c	2.20 ± 0.09a	3.84 ± 0.09b
BE01	1.24 ± 0.03f	1.48 ± 0.07d	2.7 ± 0.1e
BU01E	1.40 ± 0.05e	1.84 ± 0.02b	3.24 ± 0.03cd
Red			
RC01	1.4 ± 0.1e	1.77 ± 0.03c	3.1 ± 0.2d
RC02	1.43 ± 0.08de	1.84 ± 0.01bc	3.27 ± 0.09c
RC01E	1.42 ± 0.05e	1.88 ± 0.07b	3.3 ± 0.1c
Castellana			
CE01	1.5 ± 0.2de	0.67 ± 0.08g	2.1 ± 0.2g
CE02	1.5 ± 0.2d	0.92 ± 0.08f	2.4 ± 0.1f
CE01E	1.45 ± 0.04de	0.70 ± 0.04g	2.15 ± 0.01g
Stone			
SC01	2.78 ± 0.06a	1.06 ± 0.08b	3.8 ± 0.2a
SC02	2.1 ± 0.1b	1.09 ± 0.09e	3.2 ± 0.2cd
SE01E	2.15 ± 0.04b	1.0 ± 0.1e	3.2 ± 0.2cd

Note: Different letters in the same column indicate significant differences between means according to Tukey's HSD test ($P < 0.05$).

Table 4. Composition of organic acids of the studied Beluga, Red, Castellana, and Stone lentils (g 100 g^{-1} fw) ($n = 3$; mean ± SD)

Sample	Oxalic acid	Quinic acid	Malic acid	Shikimic acid	Total organic acids
Beluga					
BA01	1.82 ± 0.09g	3.44 ± 0.01a	0.46 ± 0.01bc	0.08 ± 0.01f	4.09 ± 0.15d
BE01	2.04 ± 0.09b	3.42 ± 0.01a	0.45 ± 0.02c	0.05 ± 0.01g	5.96 ± 0.07a
BU01E	1.19 ± 0.05f	1.31 ± 0.01g	0.62 ± 0.01a	0.04 ± 0.01h	3.17 ± 0.06h
Red					
RC01	1.30 ± 0.05e	1.86 ± 0.03e	0.37 ± 0.01d	0.11 ± 0.01cd	3.64 ± 0.01g
RC02	1.21 ± 0.06f	1.92 ± 0.01d	0.31 ± 0.01f	0.14 ± 0.01a	3.59 ± 0.08g
RC01E	1.42 ± 0.05d	2.2 ± 0.1c	0.34 ± 0.01e	0.11 ± 0.01cd	4.1 ± 0.1e
Castellana					
CE01	1.36 ± 0.05de	2.15 ± 0.01c	0.27 ± 0.01g	0.11 ± 0.01cd	3.88 ± 0.04f
CE02	1.41 ± 0.01d	2.17 ± 0.05c	0.17 ± 0.05i	0.10 ± 0.01e	3.85 ± 0.09f
CE01E	1.30 ± 0.02e	1.95 ± 0.06d	0.47 ± 0.01b	0.12 ± 0.01b	3.84 ± 0.04f
Stone					
SC01	2.32 ± 0.08a	2.29 ± 0.07b	0.25 ± 0.03h	0.11 ± 0.01d	4.96 ± 0.02b
SC02	1.81 ± 0.01c	2.29 ± 0.03b	0.31 ± 0.01f	0.10 ± 0.01e	4.50 ± 0.01c
SE01E	1.39 ± 0.01d	1.66 ± 0.01f	0.30 ± 0.02f	0.11 ± 0.01c	3.47 ± 0.04g

Note: Different letters in the same column indicate significant differences between means according to Tukey's HSD test ($P < 05$).

lipid peroxidation inhibition in porcine (*Sus scrofa*) brain homogenates and the malondialdehyde–thiobarbituric acid (MDA-TBA) complex colour strength was assessed by its absorbance at 532 nm. The inhibition ratio (%) was determined using the equation: $[(A - B)/A] \times 100\%$, where A and B are the absorbance of the control and the sample solutions, respectively.[26] The results are given as EC_{50} values (μg mL^{-1}; sample concentration providing 50% of antioxidant activity). Trolox was used as positive control.

Oxidative haemolysis inhibition assay

Anti-haemolytic activity was evaluated on the lyophilized decoction extracts through the oxidative haemolysis inhibition assay (OxHLIA) assay, as previously described.[21] An erythrocyte solution (2.8%, v/v; 200 μL) was combined with 400 μL of either extract solution (0.0938–3 mg mL^{-1} phosphate-buffered saline (PBS)), PBS (control), or water (for complete haemolysis). After pre-incubation at 37 °C for 10 min with shaking, 2,2′-Azobis (2-Amidinopropane) dihydrochloride (AAPH) (200 μL, 160 mmol L^{-1} in PBS; Sigma-Aldrich, St Louis, MO, USA) was added, and optical density was assessed at 690 nm at ~10 min intervals in a microplate reader (ELX800, Bio-Tek Instruments, Winooski, VT, USA) until full haemolysis.[27] Trolox was used as a positive control. The results were stated as IC_{50} values (μg mL^{-1}) at a increment t of 60 min, which explain the extract

concentration necessary to maintain 50% of the erythrocyte population intact for 60 min.

Statistical analysis

Twelve samples were evaluated for all the parameters, with the antioxidant assays carried out in triplicate. The results are expressed as mean values ± standard deviation. Variations between samples were evaluated using one-way analysis of variance, followed by Tukey's significant difference post hoc test with $\alpha = 0.05$, coupled with Welch's statistic. The different parameters were correlated with type of cultivation and lentil variety using a Pearson two-tailed significance correlation. IBM SPSS Statistics for Windows, version 23.0, was used (IBM Corp., Armonk, NY, USA). An unsupervised pattern recognition analysis using principal component analysis (PCA) was applied. The number of PCs for classification purposes was determined by selecting those variables with an eigenvalue >1. The data were analysed using IBM SPSS Statistics (version 27).

RESULTS AND DISCUSSION

Nutritional and energetic value

The proximate composition of the studied Beluga, Red, Castellana, and Stone lentils is presented in Table 2, with total carbohydrates standing out as the main macronutrients in all the analysed varieties, ranging from 74.4 to 77.7 g 100 g^{-1} fw. Among these, high content of starch and dietary fibre were detected, the red variety presenting the higher and lower contents of the mentioned variables (50.27% and 11.62%, respectively). This low fibre characteristic may be due to their dehulled nature since fibre is mostly present in the outer shell of the grains. On the other hand, Beluga variety presented the highest fibre content (23.13–27.36%), also exhibiting high levels of starch (41.98–46.34%). Proteins and ash arise next, with the Beluga variety showing the highest concentrations, varying from 21.9 to 23.3 g 100 g^{-1} fw for proteins, and 1.38 to 1.82 g 100 g^{-1} fw for ash. Although the Beluga lentil sample from organic farming contained the highest concentration of ash (1.82 ± 0.09 g 100 g^{-1} fw) among all samples, it was not possible to establish a correlation between this value and the type of cultivation, either with these or the other variables under investigation (Table 8). These results, together with the low-fat content (0.56–1.17 g 100^{-1} g fw), characteristic of this type of pulse, make them a valuable ingredient in low-calorie, vegan and/or vegetarian diets, among others, ensuring the supply of both micro- and macronutrients essential to human health and well-being. Moisture content and energy value showed significant differences between varieties or according to the type of cultivation. These results confirm our previous studies, in which the nutritional and chemical composition of Armuña[28] and Pardina[29] lentil varieties were analysed. In these, on average, carbohydrates were also the major macronutrient (65.03 and 66.75 g 100 g^{-1} fw, respectively), followed by proteins (23.28 and 23.05 g 100 g^{-1} fw, respectively), and with remarkable amounts of ash (2.45 and 2.61 g 100 g^{-1} fw, respectively). On the other hand, Gharibzahedi *et al.*[30] found higher percentages of protein (25.90 ± 0.14% and 27.30 ± 0.75%) and ash (3.62 ± 0.44% and 3.41 ± 0.04%), and low concentrations of fat (2.7 ± 0.31% and 2.5 ± 0.73%) in red and green Iranian lentil varieties, respectively. Similarly to our results, Tahir and colleagues[31] established that, in the Stone variety, protein concentration were, on average, 26.7 g 100 g^{-1} dry weight. Despite the small differences found between the varieties analysed in this study and the Pardina and Armuña varieties previously investigated, several factors may influence the nutritional composition of lentils, including variety, growing conditions, and edaphoclimatic factors, among others.[30] Therefore, it is not possible to establish direct and exclusive relationships between the nutritional profile and a particular isolated variable. Here, analysis of variance proved that the nutritional profile was not correlated with the type of cultivation (organic or conventional farming), presenting, however, a moderate and weak correlation between the variety and the concentration of carbohydrates and starch, and with moisture, respectively (Table 8). Tziouvalekas and colleagues[32] evaluated the seed yield, crude protein, and mineral nutrients of different lentil genotypes across diverse environments under

Table 5. Composition of tocopherols of the studied Beluga, Red, Castellana, and Stone lentils (mg 100 g^{-1} fw) ($n = 3$; mean ± SD)

Sample	γ-Tocopherol	α-Tocopherol	Total tocopherols
Beluga			
BA01	10.4 ± 0.1b	0.68 ± 0.01d	11.1 ± 0.1a
BE01	11 ± 1a	0.61 ± 0.02e	11.6 ± 1a
BU01E	9.5 ± 0.1d	0.71 ± 0.01b	10.2 ± 0.1bc
Red			
RC01	9.60 ± 0.47d	0.29 ± 0.05j	9.9 ± 0.5c
RC02	9.87 ± 0.06cd	0.69 ± 0.02d	10.56 ± 0.08b
RC01E	10.21 ± 0.08bc	0.88 ± 0.01a	11.09 ± 0.08a
Castellana			
CE01	6.96 ± 0.04g	0.60 ± 0.01e	7.57 ± 0.05e
CE02	7.5 ± 0.3ef	0.33 ± 0.03hi	7.8 ± 0.3de
CE01E	7.3 ± 0.8fg	0.39 ± 0.02g	7.6 ± 0.8e
Stone			
SC01	7.3 ± 0.8fg	0.36 ± 0.01h	7.6 ± 0.8e
SC02	7.97 ± 0.03e	0.33 ± 0.03i	8.31 ± 0.06d
SE01E	7.78 ± 0.02e	0.44 ± 0.01f	8.23 ± 0.04d

Note: Different letters in the same column indicate significant differences between means according to Tukey's HSD test ($P < 0.05$).

Table 6. Fatty acid composition of the studied Beluga, Red, Castellana, and Stone lentils (%) ($n = 3$; mean ± SD)

	Beluga			Red			Castellana			Stone		
Fatty acid	BA01	BE01	BU01E	RC01	RC02	RC01E	CE01	CE02	CE01E	SC01	SC02	SE01E
C6:0	nd	nd	nd	0.16 ± 0.01	0.19 ± 0.01	0.48 ± 0.01	0.13 ± 0.01	0.31 ± 0.01	0.14 ± 0.01	nd	nd	nd
C8:0	nd	nd	nd	0.041 ± 0.002	0.05 ± 0.01	0.15 ± 0.01	0.08 ± 0.01	0.091 ± 0.005	0.09 ± 0.01	nd	nd	nd
C11:0	0.10 ± 0.01	0.06 ± 0.01	0.043 ± 0.002	0.03 ± 0.01	0.02 ± 0.01	0.04 ± 0.01	0.18 ± 0.01	0.105 ± 0.004	0.17 ± 0.01	0.02 ± 0.01	0.07 ± 0.01	0.09 ± 0.01
C12:0	0.066 ± 0.002	0.23 ± 0.01	0.31 ± 0.01	0.16 ± 0.01	0.08 ± 0.01	0.13 ± 0.01	0.15 ± 0.01	0.28 ± 0.01	0.16 ± 0.01	nd	nd	nd
C13:0	0.203 ± 0.001	0.34 ± 0.01	0.33 ± 0.03	0.11 ± 0.01	0.11 ± 0.01	0.23 ± 0.01	0.19 ± 0.01	0.28 ± 0.01	0.18 ± 0.01	0.19 ± 0.01	0.19 ± 0.01	0.19 ± 0.01
C14:0	0.67 ± 0.06	0.71 ± 0.01	0.57 ± 0.04	0.66 ± 0.03	0.57 ± 0.01	0.66 ± 0.03	0.79 ± 0.02	0.82 ± 0.05	0.77 ± 0.02	0.95 ± 0.01	0.72 ± 0.01	0.65 ± 0.03
C14:1	0.06 ± 0.01	nd	0.37 ± 0.02	nd	nd	nd	nd	nd	nd	nd	nd	nd
C15:0	0.27 ± 0.02	0.22 ± 0.01	0.2 ± 0.1	0.2 ± 0.1	0.14 ± 0.01	0.2 ± 0.1	0.2 ± 0.5	0.31 ± 0.03	0.2 ± 0.5	0.36 ± 0.03	0.25 ± 0.01	0.26 ± 0.01
C16:0	15.1 ± 0.9	15.37 ± 0.01	15.1 ± 0.1	21 ± 2	12.1 ± 0.4	22.7 ± 0.3	16.4 ± 0.1	16.4 ± 0.9	16.6 ± 0.1	15.97 ± 0.09	16.5 ± 0.1	15.7 ± 0.2
C16:1	0.6 ± 0.1	0.32 ± 0.02	0.43 ± 0.01	0.36 ± 0.01	0.24 ± 0.01	0.54 ± 0.01	0.49 ± 0.01	0.5 ± 0.1	0.49 ± 0.01	0.28 ± 0.01	0.23 ± 0.02	nd
C17:0	0.3 ± 0.1	0.25 ± 0.01	0.24 ± 0.01	0.22 ± 0.01	0.26 ± 0.01	0.24 ± 0.01	0.27 ± 0.01	0.38 ± 0.02	0.37 ± 0.01	0.32 ± 0.02	0.25 ± 0.01	0.27 ± 0.01
C18:0	2.63 ± 0.03	2.58 ± 0.02	2.3 ± 0.1	3.08 ± 0.12	2.26 ± 0.05	3.0 ± 0.1	3.21 ± 0.04	3.31 ± 0.03	3.22 ± 0.04	3.0 ± 0.1	1.1 ± 0.1	2.4 ± 0.3
C18:1n9c	21.1 ± 0.2	22.7 ± 0.5	22.5 ± 0.5	21.40 ± 0.04	31.0 ± 0.2	21.3 ± 0.5	30.9 ± 0.2	26.4 ± 0.3	30.9 ± 0.2	20.93 ± 0.08	27.7 ± 0.1	21.2 ± 0.2
C18:2n6c	39.9 ± 0.5	40.99 ± 0.07	38.6 ± 0.6	43.8 ± 0.5	34.39 ± 0.01	41.1 ± 0.6	30.72 ± 0.01	33.4 ± 0.3	30.73 ± 0.01	39.84 ± 0.09	36.9 ± 0.1	41.0 ± 0.1
C18:3n3	0.11 ± 0.01	0.24 ± 0.01	0.13 ± 0.01	nd	nd	nd	0.09 ± 0.01	0.10 ± 0.01	0.08 ± 0.01	0.13 ± 0.01	nd	nd
C20:0	13.2 ± 0.2	10.9 ± 0.3	11.22 ± 0.05	6.3 ± 0.1b	13.6 ± 0.3	4.60 ± 0.04	10.7 ± 0.1	11.5 ± 0.2	10.6 ± 0.1	11.63 ± 0.06	10.43 ± 0.07	13.1 ± 0.1
C20:1	1.42 ± 0.04	1.4 ± 0.1	1.3 ± 0.1	1.0 ± 0.1	1.1 ± 0.1	1.3 ± 0.1	1.2 ± 0.1	1.2 ± 0.1	1.3 ± 0.1	1.78 ± 0.07	1.5 ± 0.1	1.41 ± 0.06
C20:2	1.20 ± 0.08	1.2 ± 0.1	1.8 ± 0.1	0.9 ± 0.1	1.2 ± 0.1	0.7 ± 0.1	1.0 ± 0.1	1.0 ± 0.1	1.0 ± 0.1	1.22 ± 0.01	1.18 ± 0.02	1.16 ± 0.02
C22:1	nd	0.04 ± 0.01	0.86 ± 0.01	0.05 ± 0.01	0.07 ± 0.01	0.06 ± 0.01	0.11 ± 0.01	0.12 ± 0.01	0.21 ± 0.01	0.1 ± 0.1	1.1 ± 0.1	0.06 ± 0.01
C20:5n3	1.1 ± 0.1	0.97 ± 0.05	0.95 ± 0.04	0.97 ± 0.05	1.01 ± 0.03	1.00 ± 0.02	1.02 ± 0.08	1.25 ± 0.06	1.02 ± 0.08	1.4 ± 0.1	0.2 ± 0.1	0.96 ± 0.05
C22:2	0.4 ± 0.1	0.21 ± 0.01	1.04 ± 0.03	0.18 ± 0.01	0.16 ± 0.01	0.17 ± 0.01	0.22 ± 0.01	0.26 ± 0.01	0.23 ± 0.01	nd	nd	nd
C23:0	0.05 ± 0.01	0.17 ± 0.01	0.35 ± 0.01	nd	nd	nd	nd	nd	nd	nd	nd	nd
C24:0	0.28 ± 0.01	0.3 ± 0.1	0.13 ± 0.01	0.35 ± 0.01	0.32 ± 0.02	0.29 ± 0.01	0.43 ± 0.02	0.32 ± 0.01	0.44 ± 0.02	0.38 ± 0.02	0.35 ± 0.03	0.3 ± 0.1
C24:1	0.61 ± 0.01	0.5 ± 0.1	0.74 ± 0.05	0.59 ± 0.01	0.69 ± 0.01	0.55 ± 0.01	0.90 ± 0.05	1.07 ± 0.01	0.90 ± 0.05	0.77 ± 0.02	0.70 ± 0.01	0.7 ± 0.1
SFA	32.8 ± 0.7b	31.1 ± 0.4c	30.9 ± 0.1c	32.4 ± 2.0b	29.6 ± 0.1c	32.5 ± 0.6b	32.8 ± 0.3b	34.1 ± 0.7a	33.8 ± 0.3b	32.7 ± 0.1c	29.9 ± 0.1b	32.9 ± 0.5a
MUFA	23.9 ± 0.2g	24.9 ± 0.6f	26.2 ± 0.5e	23.5 ± 0.1h	33.2 ± 0.2b	23.5 ± 0.9gh	33.8 ± 0.4a	29.4 ± 0.3d	32.8 ± 0.5g	24.0 ± 0.1c	31.4 ± 0.1h	23.4 ± 0.3f
PUFA	43.3 ± 0.5c	44.0 ± 0.2b	42.9 ± 0.5d	46.3 ± 0.6a	37.2 ± 0.1f	43.3 ± 0.6c	33.5 ± 0.3h	36.5 ± 0.4g	34.5 ± 0.2c	43.3 ± 0.1e	38.7 ± 0.1b	43.7 ± 0.2b

Note: Different letters in the same row indicate significant differences between means according to Tukey's HSD test ($P < 0.05$).
Abbreviations: nd, not detected; C6:0, caproic acid; C8:0, caprylic acid; C11:0, undecaenoic acid; C12:0, lauric acid; C13:0, tridecanoic acid; C14:0, myristic acid; C15:0, pentadecanoic acid; C16:0, palmitic acid; C16:1, palmitoleic acid; C17:0, heptadecanoic acid; C17:1, heptadecenoic Acid; C18:0, stearic acid; C18:1n9c, oleic acid; C18:2n6c, linoleic acid; C18:3n3, linolenic acid; C20:0, arachidic acid; C20:1, eicosenoic acid; C20:2, dihomo-linoleic acid; C20:4n6, arachidonic acid; C22:1, erucic acid; C20:5n3, eicosapentaenoic acid; C22:2, *cis*-13,16-docosadienoic; C23:0, tricosanoic acid; C24:0, lignoceric acid; C24:0, nervonic acid; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

organic and conventional farming. Their results showed a high influence of different environmental features on these parameters, this being the main source of micronutrient variance. Conversely, no significant differences in these parameters were observed among conventional and organic farming, as also in our study.

Chemical composition

Analysis of free sugars allowed the identification of fructose and sucrose, whose concentrations are presented in Table 3. In the Beluga and Red varieties, sucrose occurs in higher amounts (1.48–2.20 and 1.77–1.88 g 100 g^{-1} fw, respectively) than fructose (1.24–1.64 and 1.4–1.43 g 100 g^{-1} fw, respectively), although this difference is not pronounced. Conversely, in the Castellana and Stone varieties, fructose appears as the major sugar, with values ranging from 1.45 to 1.5 g 100 g^{-1} fw for the Castellana and from 2.1 to 2.78 g 100 g^{-1} fw for the Stone varieties. In these, the sucrose concentration is also relatively lower than in the Beluga and Red varieties, with values between 0.67 and 0.92 for the Castellana variety, and 1.0–1.9 for the Stone variety. Analysis of variance (Table 8) shows that the concentration of fructose is highly correlated with the variety, although no correlation was verified with the type of cultivation. In general, the type of cultivation seems to have greater effects on the chemical composition of soils, exerting positive effects on them, with very few studies proving its effect in terms of the nutritional and chemical profile of different foods.[33] These results are in part consistent with previous studies carried out on the Armuña[28] and Pardina[29] varieties, where sucrose and raffinose were identified and, in the Armuña variety, fructose was additionally noticed, as in the present study. Also, in these studies, sucrose always appears as the major sugar, the same only occurring in the Beluga and Red varieties. The differences in the profile of free sugars seem to be influenced not only by the variety but also by the different edaphoclimatic conditions to which the samples are exposed, as they come from different countries and years of cultivation, and therefore are subjected to different climatic conditions, among other features. Conceivably, for the same reasons, Tahir *et al.*,[31] besides having identified sucrose and raffinose, also noted stachyose and verbascose, these latter being frequently assigned as antinutritional factors.

Organic acid analysis allowed to identify the presence of oxalic, quinic, malic, and shikimic acids among Beluga, Red, Castellana, and Stone lentil varieties (Table 4), with quinic acid standing out as the main compound (1.31–3.44 g 100 g^{-1} fw). Among all varieties, Beluga samples from conventional production presented the highest amount of these compounds, despite not identifying any type of correlation between this concentration and the variety or type of cultivation (Table 8). Quinic acid is present in several medicinal plants, to which different biological properties have been attributed, including antibacterial, antidiabetic, and anticancer bioactivities, among others, acting through different mechanisms of action.[34] Notable amounts of oxalic acid were also detected in all samples (1.19–2.32 g 100 g^{-1} fw), followed by minor concentrations of malic and shikimic acids. The presence of organic acids in Armuña[28] and Pardina[29] lentil varieties has been investigated previously, revealing different profiles. Oxalic, shikimic and citric acids were identified in the Pardina variety, with citric acid standing out as the main compound (10.51 and 18.06 g 100 g^{-1} fw), while in the Armuña variety only oxalic and malic acids were identified, the first being the most prevalent among different samples (0.21–2.30 g 100 g^{-1} fw). Here, analysis of variance shows that concentration of oxalic and shikimic acids was moderately correlated with the variety, and that for malic acid with the type of cultivation, proving again the impact of several factors on the composition of organic compounds of lentils.

As for tocopherols, the isoforms α- and γ- were found in Beluga, Red, Castellana, and Stone lentil samples, and the results are presented in Table 5. The γ-tocopherol isoform was highly prevalent among all varieties, presenting values ranging from 7.25 to 10.98 mg 100 g^{-1} fw. Although, once again, the Beluga variety, and specifically the samples belonging to conventional farming, showed the highest concentration of this isoform, analysis of variance did not show any association between this and the type of cultivation and variety (Table 8). A similar pattern was previously identified in Armuña[28] and Pardina[29] lentil varieties, with γ-tocopherol isoform presenting the highest average concentration in both varieties (3.37 and 7.95 mg 100 g^{-1} fw, respectively), though being higher in the present study (9.11 mg 100 g^{-1} fw, on average). Similarly, Padhi *et al.*[35] investigated the presence of tocopherols in split red, small and large green Canadian lentils, also identifying α- and γ-tocopherol isoforms, with the last being predominant among the analysed varieties. The occurrence of the γ-tocopherol isoform in different products has been associated with higher levels of antioxidant, anti-inflammatory, and other bioactivities, and may therefore be associated with the prevention of various acute and chronic disorders.[36]

Investigation of the FAME profile of Beluga, Red, Castellana, and Stone lentil varieties allowed the identification of 26 different compounds (Table 6), of which C18:2n6c (linoleic acid, 30.72–43.8%) stood out as the most prevalent in all samples, followed by C18:1n9c (oleic acid, 21.13–31.04%), C16:0 (palmitic acid, 12.1–22.7%), and C20:0 (eicosanoic acid, 4.60–13.55%). Considering the classification, polyunsaturated fatty acids (PUFA) were present in greater amounts in all samples (33.5–46.3%), followed by saturated fatty acids (SFA, 29.6–34.1%), and finally monounsaturated fatty acids (MUFA, 23.4–33.8%).

Table 7. Antioxidant activity of the studied Beluga, Red, Castellana, and Stone lentils ($n = 3$; mean ± SD)

Sample	TBARS (EC_{50}; μg/mL)	OxHLIA (IC_{50}; μg/mL); $\Delta t = 60$ min
Beluga		
BA01	56 ± 4d	106 ± 3h
BE01	53 ± 1de	147 ± 5ef
BU01E	42 ± 2de	130 ± 4fg
Red		
RC01	48 ± 3de	404 ± 12a
RC02	57 ± 7d	326 ± 16c
RC01E	97 ± 10c	384 ± 19b
Castellana		
CE01	40 ± 7e	166 ± 5d
CE02	20 ± 12f	124 ± 5g
CE01E	53 ± 4de	162 ± 7de
Stone		
SC01	773 ± 49a	149 ± 7e
SC02	769 ± 24a	97 ± 7h
SE01E	203 ± 11b	90 ± 6h

Note: EC_{50}: extract concentration corresponding to 50% antioxidant activity. Trolox EC_{50} values: 23 μg mL^{-1} (TBARS inhibition) and 21.8 μg mL^{-1} (OxHLIA $\Delta t = 60$ min); Different letters in the same column indicate significant differences between means according to Tukey's HSD test ($P < 0.05$).

These results confirm our previous study with Pardina lentils,[29] in which SFA were present in greater quantities than MUFA, mainly due to the presence of palmitic acid, while in Armuña lentils[28] the latter were found in higher percentages than SFA.

Table 8. Correlation coefficients among nutritional, chemical composition, antioxidant capacity, variety, and type of cultivation of the studied Beluga, Red, Castellana, and Stone lentils

Parameter	Variety	Type of cultivation
Moisture (%)	0.188*	−0.375
Fat (g 100 g^{-1} fw)	−0.337	0.04
Proteins (g 100 g^{-1} fw)	−0.493	0.022
Ash (g 100 g^{-1} fw)	−0.096	−0.028
Fiber (%)	−0.414	0.012
Starch (%)	0.446**	−0.284
Carbohydrates (g 100 g^{-1} fw)	0.575**	−0.017
Energy value (kcal 100 g^{-1} fw)	−0.168	0.017
Fructose (g 100 g^{-1} fw)	0.728****	0.097
Sucrose (g 100 g^{-1} fw)	−0.570	−0.100
Total sugars (g 100 g^{-1} fw)	0.46**	−0.137
Oxalic acid (g 100 g^{-1} fw)	0.480**	−0.109
Quinic acid (g 100 g^{-1} fw)	−0.339	−0.502
Malic acid (g 100 g^{-1} fw)	−0.678	0.443**
Shikimic acid (g 100 g^{-1} fw)	0.564**	−0.051
Total organic acids (g 100 g^{-1} fw)	−0.030	−0.439
α-Tocopherol (mg 100 g^{-1} fw)	−0.630	0.308**
γ-Tocopherol (mg 100 g^{-1} fw)	−0.827	−0.049
Total tocopherols (mg 100 g^{-1} fw)	−0.840	−0.008
SFA (%)	0.145*	0.063
MUFA (%)	0.295*	−0.370
PUFA (%)	−0.371	0.324**
TBARS	0.657****	−0.227
OxHLIA	0.275*	0.007

Note: Asterisks indicate *negligible, **weak, ***moderate, and ****strong correlation.

Furthermore, in both previous studies, α-linolenic acid (C18:3n3) was identified in high ratios (12.79–15.85% and 9.6–15.1%, respectively), although this was not verified in the present investigation, where values ranged from 0.08% to 0.24%. Despite that, analysis of variance showed a weak correlation between the percentage of SFA and MUFA with variety, and a moderate correlation of PUFA with the type of cultivation (Table 8). Similarly, Padhi *et al.*[35] and Zhang *et al.*[37] found comparable FAME profiles in red and green Canadian lentils, with linoleic acid the major compound in all varieties, followed by oleic acid. Despite these similarities, in both studies PUFA were the major group of FAME present in all samples, followed by MUFA, which is not in accordance with the present investigation, in which PUFA were followed by higher percentages of SFA. These differences may be related to different features such as the variety, country of origin, and edaphoclimatic conditions, which may enhance the synthesis of certain compounds according to the needs of the experiment matrix itself.

Antioxidant activity evaluation

The antioxidant activity of Beluga, Red, Castellana, and Stone lentil varieties were assessed using the decocted lyophilized extracts of each sample and two *in vitro* assays that measure the ability to avoid lipid peroxidation of porcine brain tissues (TBARS) and haemolysis of sheep blood cells (OxHLIA). The results, presented in Table 7, show that Beluga, Red, and Castellana lentil varieties extracts demonstrated a very good antioxidant capacity through the TBARS assay, with Castellana presenting the most promising results (EC_{50} = 20–53 μg mL^{-1}). Inversely, extracts from Stone variety showed a weak antioxidant capacity, presenting high EC_{50} values, between 203 and 773 μg mL^{-1}. In contrast, in the OxHLIA assay, extracts from the Stone variety presented the best results (IC_{50} = 90–149 μg mL^{-1}), followed by Beluga (IC_{50} = 106–147 μg mL^{-1}) and

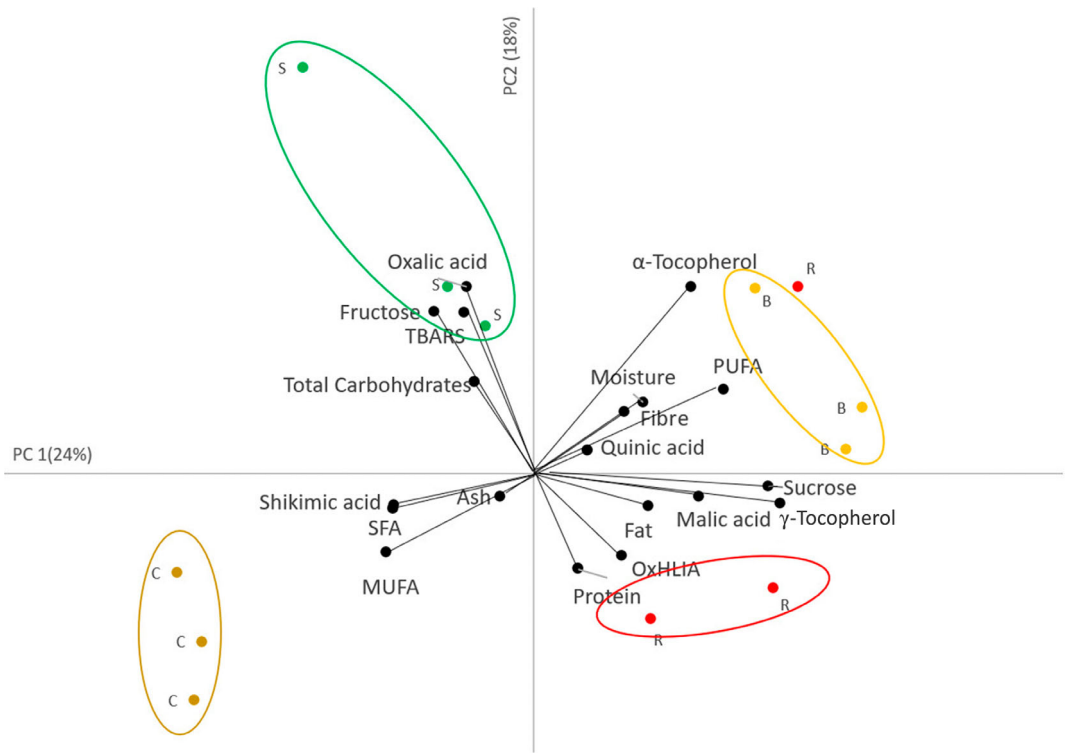

Figure 1. Projection plot of lentil samples in the space defined by the two firsts PCs. C, Castellana variety; S, Stone variety; R, Red variety; B, Beluga variety.

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Castellana (IC_{50} = 124–166 μg mL^{-1}) variety extracts. These divergences may be related to the way different antioxidant assays act on different types of free radicals and their interaction with living organisms, making it difficult to choose a single method that best describes the antioxidant ability of a given sample.[38] Here, the analysis of variance shows a strong correlation between variety and TBARS, while a weak correlation was found between the TBARS and OxHLIA assays. Also, no correlations were found between either assay and type of cultivation. The antioxidant capacity of lentils was also investigated previously by our group, with the results showing a weaker capacity performed by the decocted extracts of Pardina lentils (EC_{50} = 211–228 μg mL^{-1} for TBARS; IC_{50} = 63–809 μg mL^{-1} for OxHLIA) and with values in a similar range to the present study for Armuña lentils (EC_{50} = 30–99 μg mL^{-1} for TBARS; IC_50 = 67–673 μg mL^{-1} for OxHLIA).[22,23] Overall, the good antioxidant capacity of lentils seems to be related to their notable chemical profile, namely through the presence of different organic acids and tocopherols. Over the years, the antioxidant capacity of lentil extracts has been investigated using a broad range of different methods,[39-41] with the results agreeing with those in the present study.

Correlation coefficients and multivariate statistical analysis

Pearson's correlation coefficient has been applied to evaluate possible correlations between the different parameters analysed and the lentil variety or cultivation type. Table 8 shows the correlations obtained. Lentil variety is strongly correlated with fructose concentration and with TBARS antioxidant activity. All other correlations observed for both variety (Beluga, Castellana, Stone, and Red) and cultivation type (conventional *vs*. organic) are weak.

It is relevant to note that Pearson's correlation only shows the occurrence of a linear relationship between two variables. In practice, it is difficult for a food compositional parameter to show linear relationships with variables such as cultivation type or variety. Therefore, the overall variation and the relationships between the different variables were investigated using PCA. Being an unsupervised learning method, PCA analysis allows us to identify groups using the variables analysed and is a very suitable tool for data visualisation. For the analysis, the variables were normalised, scaled and mean-centred. Forty-two of the variables analysed in this study were used for the PCA analysis. Those variables that, for all the samples analysed, did not obtain a value higher than 0 were removed. In total eight variables were eliminated, corresponding to eight fatty acids (Table 6). PCA yielded nine PCs with an eigenvalue > 1, explaining 96.218% of the variance. Figure 1 shows the projection diagram of the samples and the original variables in the space defined by the first two PCs. PC1, which is the most important component, accounts for 24.69% of the total variation and is positively related to sucrose (0.887) and γ-tocopherol (0.931), and negatively correlated with C24:1 fatty acid (−0.851). PC2 accounted for 18.02% of the total variation and is positively correlated with fructose (0.868) and TBARS (0.865), and negatively correlated with C16:1 (−0.525) and protein (−0.515).

Figure 1 shows a clear grouping of the analysed samples according to lentil variety; only one of the red lentil samples is shown outside this grouping due to large differences in content compared to the other two red lentil samples. Regarding the organic or conventional production, there is no pattern of varieties grouped together. Beluga lentils were found in the upper right quadrant with positive values for both PCs, due to the higher fibre, sucrose, and tocopherol content of this variety. The red lentil samples had positive values for PC1 and negative values for PC2; these values correlated with their antioxidant activity in OxHLIA assay. The lentil varieties Castellana and Stone had negative PC1 values, related to the lower tocopherol concentrations of these varieties. The fructose content of the Stone variety placed it within the positive PC2 values.

CONCLUSIONS

Different varieties of lentils consumed and appreciated worldwide, namely the Beluga, Red, Castellana, and Stone varieties, were analysed for their nutritional, chemical, and antioxidant potential. The results showed the prevalence of carbohydrates in all varieties, of which notable proportions of starch and fibre were identified. Also, high amounts of proteins and ash were detected in all samples, mainly in the Beluga variety. Chemical analysis allowed the identification of fructose and sucrose as free sugars, as well as several organic acids, with the prevalence of quinic acid in all varieties, and α- and γ-tocopherol isoforms. From FAME assessment, PUFA appeared as the major compounds, with the prevalence of linoleic (C18:2n6) and oleic (C18:1n9) acids in all samples. Good antioxidant activity was also detected in the decocted extracts of all varieties, except for Stone, which presented EC_{50} values far above the other varieties. Analysis of variance showed a mostly negligible or weak correlation of the attained results with the type of cultivation (conventional and organic), and a much stronger correlation of these with the variety.

Together, these results make lentils an excellent ingredient in various diets, such as vegan/vegetarian, low-calorie, and low-glycaemic diets, supporting their regular consumption as a strategy to promote human health and well-being. Additionally, the weak correlation of the analysed parameters with the type of cultivation needs further investigation in these and other experiment matrices, to better clarify the possible and dubious impact of the type of cultivation on the nutritional and chemical profiles of different ingredients.

AUTHOR CONTRIBUTIONS

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 and editing, supervision. Carla Pereira: methodology, data curation. Isabel CFR Ferreira: project administration. Ana María Vivar-Quintana: conceptualization, visualization, supervision, writing – review and editing. Lillian Barros: validation, investigation, writing – review and editing, visualization, supervision, project administration, funding acquisition. All authors have read and agreed to the published version of the manuscript.

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CONFLICT OF 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 STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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