Valorisation of traditional foods: nutritional and bioactive properties of *Cicer arietinum* L. and *Lathyrus sativus* L. pulses

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Running title: Valorisation of traditional foods

Abstract

Background: The use of traditional foods can enrich our diet, perpetuating important elements of local knowledge and cultural inheritance. Raw, soaked and cooked samples of two Fabaceae species (*Cicer arietinum* L. and *Lathyrus sativus* L.) were characterized regarding nutritional and bioactive properties.

Results: *L. sativus* gave the highest carbohydrate, protein, ash, SFA and PUFA content, and lowest fat and energy value. Furthermore, it also showed the highest concentration in flavonoids and antioxidant activity. *C. arietinum* gave the highest concentration of sugars, organic acids and tocopherols. Soaking process did not affect significantly macronutrients, but cooking (boiling) decreased protein, ash, sugars and organic acids, and increased carbohydrates, fat, tocopherols, bioactive compounds and antioxidant activity. No differences were obtained for fatty acids composition.

Conclusion: The present study highlights the nutritional profile and bioactive properties of these farmer varieties of *C. arietinum* and *L. sativus* pulses, and valorises their traditional consumption and the use in modern diets.

Keywords *Cicer arietinum*; *Lathyrus sativus*; Traditional foods; Chromatography; Nutritional; Bioactivity

Introduction

The use of traditional foods can enrich and improve our diet and at the same time perpetuate important elements of local knowledge and cultural heritage.[1,2] They are key elements for the dietary patterns in different countries and consequently are important to accurately estimate population dietary intakes.[3] However, throughout Europe, traditional foods are threatened with extinction due to altered lifestyles[2] and nutritional information of these foods is missing from most current food composition databases.[3]

Otherwise, consumers are looking for new foods with different organoleptic characteristics to those routinely consumed every day at home. Traditional foods may contribute to this demand, which are often perceived as higher quality and more sustainable foods that fulfil a need for cultural identity and ethnocentrism.[4]

Good examples of traditional foods are the Portuguese farmer varieties of two species of Fabaceae, *Cicer arietinum* L. and *Lathyrus sativus* L. pulses (edible seeds within a pod), cultivated since immemorial times in a specific region of Northeastern Portugal, so called Planalto Mirandês. The seeds were kept for generations circulating among neighbours and family being eventually commercialized in local markets, but they are not available in seeds official distribution circuits.[5]

C. arietinum (chickpea) is considered to be a healthy vegetarian food, being also one of the oldest and most widely consumed (by human and domestic animals) legumes in the world including different countries of Asia, Africa, Europe, Middle East, North and South America.[6-8] It is a cheap source of high quality protein in the diets of millions in developing countries, who cannot afford animal protein for balanced nutrition. It is also a good source of carbohydrates, minerals and trace elements.[9] Besides nutrition, chickpea also has antioxidant phytochemicals and activity that could contribute to the

prevention of ageing, cancer, neurodegenerative and cardiovascular diseases, when properly processed.[10-11]

L. sativus (white pea) is probably the oldest crop cultivated in Europe.[12] It is widely consumed in developing countries of Mediterranean area, Asia and Africa, being as the previous mentioned species a good source of proteins, carbohydrates[13,14] and antioxidant compounds.[15,16] Nevertheless, its consumption demands special procedures due to the presence of neurotoxic amino acids related to neurolathyrism.[14]

The nutritional value of pulses is determined by the content of biologically available nutrients and the effects of anti-nutrients such as trypsin inhibitors, phytic acid, tannins and oligosaccharides (raffinose, stachyose and verbascose) that limit protein and carbohydrates utilization.[8] Heat treatment significantly improves pulses protein quality by destruction or inactivation of heat labile anti-nutritional factors. However, cooking can also affect their chemical composition, causing considerable losses in soluble solids, especially vitamins and minerals,[6,8] and antioxidant properties.[17]

The present study intends to valorise traditional foods (raw, soaked and cooked Portuguese farmer varieties of *C. arietinum* and *L. sativus* seeds) through dissemination of their use, highlighting the nutritional value and bioactive properties.

Material and Methods

Samples and samples preparation

Cicer arietinum L. and *Lathyrus sativus* L. were important crops in former times and a safeguard against hunger, strengthening local food systems and environmental sustainability. Besides their use as fodder, immature green pods, seeds and flour were

consumed by rural people and a source of nutrients that improved dietary diversity and quality.

From both species, mature seeds produced in 2011, were assigned by an informant from Picote, Miranda do Douro, Portugal, who is a renowned producer and seed guardian. Morphological key characters from the Flora Iberica[18] for *L. sativus* and from Kew database Plants & Fungi[19] for *C. arietinum* were used for identification and nomenclature. Voucher specimens are deposited in the Escola Superior Agrária de Bragança herbarium (BRESA).

Samples for analysis were prepared considering the culinary use of the seeds, which presupposes making them into powder (flour) or soaking them for 24 hours before cooking (boiling) and being used as main ingredient in traditional recipes. To achieve our goal, three different samples were made: (i) raw sample, mature seeds that were ground; (ii) soaked sample, mature seeds soaked for 24 hours in distilled water and then ground; (iii) cooked (boiled) sample, mature seeds pre-soaked as explained above, cooked also in distilled water for 15 minutes employing a pressure cooker and then ground for analysis.

Standards and reagents

Acetonitrile 99.9%, n-hexane 95% and ethyl acetate 99.8% were of HPLC grade from Lab-Scan (Lisbon, Portugal). The fatty acids methyl ester (FAME) reference standard mixture 37 (standard 47885-U) was purchased from Sigma (St. Louis, MO, USA), as also other individual fatty acid isomers, L-ascorbic acid, tocopherol, organic acid and sugar standards, trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), gallic acid and (+)-catechin. Racemic tocol, 50 mg/ml, was purchased from Matreya

(PA, USA). 2,2-Diphenyl-1-picrylhydrazyl (DPPH) was obtained from Alfa Aesar (Ward Hill, MA, USA). All other chemicals and solvents were of analytical grade and purchased from common sources. Water was treated in a Milli-Q water purification system (TGI Pure Water Systems, USA).

Nutritional value

Macronutrients. The samples were analysed for chemical composition (moisture, protein, fat, carbohydrates and ash) using the AOAC procedures.[20] The crude protein content (N × 6.25) of the samples was estimated by the macro-Kjeldahl method; the crude fat was determined by extracting a known weight of powdered sample with petroleum ether, using a Soxhlet apparatus; the ash content was determined by incineration at 600±15 ºC. Total carbohydrates were calculated by difference. Total energy was calculated according to the following equations: Energy (kcal) = 4 × (g protein +g carbohydrate) + 9 × (g lipid).

Fatty Acids. Fatty acids were determined by gas-liquid chromatography with flame ionization detection (GC-FID)/capillary column as described previously by the Barros *et al*.[21] and after the following trans-esterification procedure: fatty acids (obtained after Soxhlet extraction) were methylated with 5 mL of methanol:sulphuric acid:toluene 2:1:1 (*v:v:v*), during at least 12 h in a bath at 50 ºC and 30 *g*; then 3 mL of deionised water were added, to obtain phase separation; the FAME were recovered with 3 ml of diethyl ether by shaking in vortex , and the upper phase was passed through a micro-column of sodium sulphate anhydrous, in order to eliminate the water; the sample was recovered in a vial with Teflon, and before injection the sample was filtered with 0.2 μm nylon filter

from Whatman. The analysis was carried out with a DANI model GC 1000 instrument equipped with a split/splitless injector, a flame ionization detector (FID at 260 ºC) and a Macherey–Nagel (Düren, Germany) column (50% cyanopropyl-methyl-50% phenylmethylpolysiloxane, 30 m × 0.32 mm i.d. × 0.25 μm d_f). The oven temperature program was as follows: the initial temperature of the column was 50 ºC, held for 2 min, then a 30 ºC/min ramp to 125 ºC, 5 ºC/min ramp to 160 ºC, 20 ºC/ min ramp to 180 ºC, 3 ºC/min ramp to 200 ºC, 20 ºC/min ramp to 220 ºC and held for 15 min. The carrier gas (hydrogen) flow-rate was 4.0 mL/min (0.61 bar), measured at 50 ºC. Split injection (1:40) was carried out at 250 ºC. Fatty acids identification was made by comparing the relative retention times of FAME peaks from samples with standards. The results were recorded and processed using the Clarity DataApex 4.0 Software and expressed in relative percentage of each fatty acid.

Sugars. Free sugars were determined by high performance liquid chromatography coupled to a refraction index detector (HPLC-RI) as described by Barros *et al.*[21] Dried sample powder (1 g) was spiked with the melezitose as internal standard (IS, 5 mg mL^{-1}), and was extracted with 40 mL of 80% aqueous ethanol at 80 ºC for 30 min. The resulting suspension was centrifuged (Centurion K24OR refrigerated centrifuge, West Sussex, UK) at 15,000*g* for 10 min. The supernatant was concentrated at 60 ºC under reduced pressure and defatted three times with 10 mL of ethyl ether, successively. After concentration at 40 ºC, the solid residues were dissolved in water to a final volume of 5 mL and filtered through 0.2 μm nylon filters from Whatman. The equipment of analysis consisted of an integrated system with a pump (Knauer, Smartline system 1000, Brelin, Germany), degasser system (Smartline manager 5000), auto-sampler (AS-2057 Jasco,

Easton, MD) and an RI detector (Knauer Smartline 2300). Data were analysed using Clarity 2.4 Software (DataApex). The chromatographic separation was achieved with a Eurospher 100-5 NH_2 column (4.6 × 250 mm, 5 mm, Knauer) operating at 30 ºC (7971 R Grace oven). The mobile phase was acetonitrile/deionized water, 70:30 (*v/v*) at a flow rate of 1 mL min^{-1}. The compounds were identified by chromatographic comparisons with authentic standards. Quantification was performed using the internal standard method and sugar contents were further expressed in g kg^{-1} of dry weight.

Organic acids. Organic acids were determined following a procedure previously described by the authors.[22] Samples (~2 g) were extracted by stirring with 25 mL of meta-phosphoric acid (25ºC at 30 *g*) for 45 min and subsequently filtered through Whatman No. 4 paper. Before analysis, the sample was filtered through 0.2 μm nylon filters. The analysis was performed using a Shimadzu 20A series UFLC (Shimadzu Corporation, Kyoto, Japan). Separation was achieved on a SphereClone (Phenomenex) reverse phase C_{18} column (5 μm, 250 mm × 4.6 mm i.d.) thermostatted at 35 ºC. The elution was performed with sulphuric acid 3.6 mM using a flow rate of 0.8 mL min^{-1}. Detection was carried out in a PDA, using 215 nm and 245 nm (for ascorbic acid) as preferred wavelengths. The organic acids found were quantified by comparison of the area of their peaks recorded at 215 nm with calibration curves obtained from commercial standards of each compound. The results were expressed in g kg^{-1} of dry weight.

Tocopherols. Tocopherols content was determined following a procedure previously optimized and described by Barros *et al*.[21] BHT solution in hexane (10 mg mL^{-1}; 100

μL) and IS solution in hexane (tocol; 50 μg mL^{-1}; 400 μL) were added to the sample prior to the extraction procedure. The samples (~500 mg) were homogenized with methanol (4 mL) by vortex mixing (1 min). Subsequently, hexane (4 mL) was added and again vortex mixed for 1 min. After that, saturated NaCl aqueous solution (2 mL) was added, the mixture was homogenized (1 min), centrifuged (5 min, 4000*g*) and the clear upper layer was carefully transferred to a vial. The sample was re-extracted twice with hexane. The combined extracts were taken to dryness under a nitrogen stream, redissolved in 2 mL of n-hexane, dehydrated with anhydrous sodium sulphate, filtered through 0.2 μm nylon filters from Whatman, transferred into a dark injection vial and analysed by the HPLC system described above connected to a fluorescence detector (FP-2020; Jasco) programmed for excitation at 290 nm and emission at 330 nm. The chromatographic separation was achieved with a Polyamide II (250 mm × 4.6 mm i.d.) normal-phase column from YMC Waters operating at 30 ºC. The mobile phase used was a mixture of n-hexane and ethyl acetate (70:30, *v/v*) at a flow rate of 1 mL/min, and the injection volume was 20 μL. The compounds were identified by chromatographic comparisons with authentic standards. Quantification was based on calibration curves obtained from commercial standards of each compound using the IS methodology. The results were expressed in mg kg^{-1} of dry weight.

Bioactivity

Extracts preparation. The extractions were performed using a fine dried powder (20 mesh; ~10 g) stirring with 50 mL of methanol at 25 ºC at 30 *g* for 1 h and filtered through Whatman No. 4 paper. The residue was then extracted with one additional 50 mL portion of methanol. The combined methanolic extracts were evaporated at 35ºC

under reduced pressure (rotary evaporator Büchi R-210) and re-dissolved in methanol at a known concentration.

Bioactive compounds. Phenolics were estimated based on procedures described by Wolfe *et al.*[23] with some modifications. An aliquot of the extract solution (1 mL) was mixed with *Folin-Ciocalteu* reagent (5 mL, previously diluted with water 1:10 *v/v*) and sodium carbonate (75 g L^{-1}, 4 mL). The tubes were vortexed for 15 s and allowed to stand for 30 min at 40 °C for colour development. Absorbance was then measured at 765 nm. Gallic acid was used to calculate the standard curve and the results were expressed as mg of gallic acid equivalents (GAE) per g of extract.

Flavonoids were determined using the method of Jia *et al.*[24], with some modifications. An aliquot (0.5 mL) of the extract solution was mixed with distilled water (2 mL) and subsequently with $NaNO_2$ solution (5%, 0.15 mL). After 6 min, $AlCl_3$ solution (10%, 0.15 mL) was added and allowed to stand further 6 min, thereafter, NaOH solution (4%, 2 mL) was added to the mixture. Immediately, distilled water was added to bring the final volume to 5 mL. Then the mixture was properly mixed and allowed to stand for 15 min. The intensity of pink colour was measured at 510 nm. (+)-Catechin was used to calculate the standard curve and the results were expressed as mg of (+)-chatequin equivalents (CE) per g of extract.

DPPH radical-scavenging activity. This methodology was performed using an ELX800 Microplate Reader (Bio-Tek, Bedfordshire, UK). The reaction mixture in each one of the 96-wells consisted of one of the different concentration solutions (30 μL) and methanolic solution (270 μL) containing DPPH radicals (6×10^{-5} mol L^{-1}). The mixture

was left to stand for 30 min in the dark. The reduction of the DPPH radical was determined by measuring the absorption at 515 nm. The radical scavenging activity (RSA) was calculated as a percentage of DPPH discolouration using the equation: RSA (%) = $[(A_{DPPH}-A_S)/A_{DPPH}] \times 100$, where A_S is the absorbance of the solution when the sample extract has been added at a particular level, and A_{DPPH} is the absorbance of the DPPH solution.[21] The extract concentration providing 50 % of antioxidant activity (EC_{50}) was calculated from the graph of DPPH scavenging activity against extract concentrations. Trolox was used as a standard.

Reducing power. This methodology was performed using the Microplate Reader described above. The different concentration solutions (0.5 mL) were mixed with sodium phosphate buffer (200 mmol L^{-1}, pH 6.6, 0.5 mL) and potassium ferricyanide (1 % *w/v*, 0.5 mL). The mixture was incubated at 50 ºC for 20 min, and trichloroacetic acid (10 % *w/v*, 0.5 mL) was added. The mixture (0.8 mL) was poured in the 48-wells, as also deionised water (0.8 mL) and ferric chloride (0.1 % *w/v*, 0.16 mL), and the absorbance was measured at 690 nm.[21] The extract concentration providing 0.5 of absorbance (EC_{50}) was calculated from the graph of absorbance at 690 nm against extract concentrations. Trolox was used as a standard.

Inhibition of β-carotene bleaching. A solution of β-carotene was prepared by dissolving β-carotene (2 mg) in chloroform (10 mL). Two millilitres of this solution were pipetted into a round-bottom flask. After the chloroform was removed at 40 ºC under vacuum, linoleic acid (40 mg), Tween 80 emulsifier (400 mg), and distilled water (100 mL) were added to the flask with vigorous shaking. Aliquots (4.8 mL) of this emulsion were

transferred into different test tubes containing different concentrations of the samples (0.2 mL). The tubes were shaken and incubated at 50 ºC in a water bath. As soon as the emulsion was added to each tube, the zero time absorbance was measured at 470 nm in a spectrophotometer (AnalytikJena, Jena, Germany). β-Carotene bleaching inhibition was calculated using the following equation: (Abs after 2h of assay/initial Abs) × 100.[21] The extract concentration providing 50% of antioxidant activity (EC_{50}) was calculated from the graph of β-carotene bleaching inhibition against extract concentrations. Trolox was used as a standard.

Inhibition of lipid peroxidation using thiobarbituric acid reactive substances (TBARS). Porcine (*Sus scrofa*) brains were obtained from official slaughtering animals, dissected, and homogenized with a Polytron in ice-cold Tris–HCl buffer (20 mM, pH 7.4) to produce a 1:2 (*w/v*) brain tissue homogenate, which was centrifuged at 3000*g* for 10 min. An aliquot (0.1 mL) of the supernatant was incubated with the different solution concentrations (0.2 mL) in the presence of $FeSO_4$ (10 μM; 0.1 mL) and ascorbic acid (0.1 mM; 0.1 mL) at 37 ºC for 1 h. The reaction was stopped by the addition of trichloroacetic acid (28 % *w/v*, 0.5 mL), followed by thiobarbituric acid (TBA, 2 %, *w/v*, 0.38 mL), and the mixture was then heated at 80 ºC for 20 min. After centrifugation at 3000*g* for 10 min to remove the precipitated protein, the colour intensity of the malondialdehyde (MDA)-TBA complex in the supernatant was measured by its absorbance at 532 nm. The inhibition ratio (%) was calculated using the following formula: Inhibition ratio (%) = [(A – B)/A] × 100 %, where A and B were the absorbance of the control and the compound solution, respectively.[21] The extract concentration providing 50% of antioxidant activity (EC_{50}) was calculated from the

graph of TBARS formation inhibition against extract concentrations. Trolox was used as a standard.

Statistical analysis

All the assays were carried out in triplicate and the results are expressed as mean values and standard deviation (SD). The results were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's HSD Test with $\alpha = 0.05$, performed with SPSS v. 18.0 program.

Results and Discussion

Nutritional value

The results of the macronutrients and energetic value obtained for *C. arietinum* and *L. sativus* seeds are shown in **Table 1**. Carbohydrates were the predominant macronutrients and were higher in *L. sativus* than in *C. arietinum*. The values found in raw *L. sativus* were similar to the ones reported for Canadian and Indian varieties (635 and 643 g kg^{-1} dw, respectively.[13] Carbohydrates include fibre and it would be interesting to determine, separately, soluble and insoluble fibre contents. Protein was the second component most abundant and followed the same tendency. The content observed in raw *L. sativus* was higher than protein levels described for Polish (275 g kg^{-1}),[25] Canadian and Indian samples (236 and 213 g kg^{-1} dw, respectively).[13] Ash content was also higher in the same species, and the values observed were, once more, higher than the ones reported for Polish (313 g kg^{-1})[25], Canadian and Indian samples (29 and 27 g kg^{-1} g dw, respectively).[13] The protein and ash contents found in *C. arietinum* raw sample were similar to the ones reported by Amir *et al.*[26] for Algerian sample, but

protein content was higher than the value reported by Fares and Menga[17] for *C. arietinum* flour (234 g kg^{-1} dw).

Despite the tendency observed for the other macronutrients, it was *C. arietinum* that showed the highest fat levels and energetic contribution. Moreover, its raw sample gave higher fat content than Canadian and Indian varieties (13 and 12 g kg^{-1} dw, respectively.[13]

In general, there were no statistically significant differences between macronutrients in raw and soaked samples. The only exceptions were fat content that increased from raw to soaked samples of both species, and energetic contribution that also increased in the case of *C. arietinum*. Nevertheless, significant differences were obtained among raw and cooked samples, with a decrease in ash and proteins content, and an increase in fat, carbohydrates and energy values (**Table 1**).

Despite the existence of some studies comparing raw and cooked samples of *C. arietinum*, the results are not conclusive. Bhatty *et al.*[27] observed, like our research group, a loss in protein during cooking due to degradation processes. Wang *et al.*[8] described an increase in protein content attributed to the loss of soluble solids during cooking, while Alajaji and El-Adawy[6] reported similar values among raw and boiled samples. Regarding ash content, both authors observed a decrease in cooked samples, which is in agreement with the present study, explained by diffusion of certain minerals into the cooking water.[8] The effects on fat were also dissimilar according to each author; Alajaji and El-Adawy[6] described a decrease, while Wang *et al.*[8] observed a significant increase, like in the present study. As far as we know, there are not available reports on the effects of soaking and cooking on macronutrients of *L. sativus*.

The results for fatty acid composition, total saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA) of the studied seeds are shown in **Table 2**. Linoleic acid (C18:2n6), oleic acid (C18:1n9) and palmitic acid (C16:0) were the main polyunsaturated, monounsaturated and saturated fatty acids respectively, and their abundance was in the order presented. The hypocholesterolaemic effect of *C. arietinum* has been related to its high content of essential fatty acids, mainly linoleic acid.[28] Both species revealed PUFA>MUFA>SFA contents.

C. arietinum showed higher MUFA levels than *L. sativus*, but the latter species gave higher SFA and also slightly higher PUFA contents. Soaking and cooking processed did not change significantly the fatty acids profile or amounts in both species; this is in agreement with Attia *et al.*[28] that reported only a slight change in fatty acids composition of *C. arietinum* with cooking. Nevertheless, some differences could be observed in its raw sample and samples from Algeria[26] and Pakistan[9]: lower palmitic and arachidic acids, and higher α-linolenic acid. The profile and amounts of fatty acids found in *L. sativus* raw sample were very similar to the ones described for a Canadian variety.[13] Nonetheless, it presented differences regarding a sample from Poland[25], namely in palmitic and linoleic acids content. These variations can be either due to intrinsic factors (mainly genetics, which are partly responsible for differences between cultivars and varieties) or to extrinsic factors, such as storage, type of soil, agronomic practices, climatic factors and technological treatments.[9]

The sugars composition is presented in **Table 3**. *C. arietinum* presented a higher concentration and a more diverse profile in sugars (sucrose> stachyose> verbascose> raffinose> fructose> trehalose) then *L. sativus* that only presented sucrose. There are no

studies in this last sample regarding sugars composition. Samples of *C. arietinum* from Kingdom of Saudi Arabia[6] and China[29] presented similar sugars profile and contents, but without trehalose. Nonetheless, Xiaolia *et al.*[29] isolated and identified another sugar, ciceritol, as main compound. It was observed a reduction in sugar contents from raw to cooked samples (**Table 3**); this was also demonstrated in samples of *C. arietinum* from Kingdom of Saudi Arabia[6] and Canada[8] and could probably be explained by their diffusion into the cooking water.

Three organic acids (oxalic, malic and citric acids) were identified in both samples (**Table 3**) and *C. arietinum* presented the highest amount. A reduction in all the organic acids found in both species was observed after soaking and cooking procedures; this could be due to a degradation/oxidation process. As far as we know, there are no reports describing organic acids in these samples or presenting the effect of cooking. Ascorbic acid was also absent in the studied samples, which is in agreement with the described by Chavan et al.[13] in a *L. sativus* sample from Canada.

Tocopherols content is present in **Table 3**; *C. arietinum* presented the highest amounts of tocopherols and presented all isoforms. *L. sativus* only presented α- and γ-tocopherols. Grela and Gunter[25] identified three isoforms (α-, γ- and δ-tocopherols) in *L. sativus* from Poland and revealed a higher amounts in all isoforms. Aslam *et al.*[7] also identified α-tocopherol in a sample of *C. arietinum* from India, but in lower content. All isoforms and total content of tocopherols increased in both species with the soaking and cooking procedures, probably due to a higher extractability of this vitamin. We could not find studies regarding the effects of cooking procedures on tocopherols content.

Bioactivity

Analyzing the results presented for bioactive compounds (**Table 4**), both species revealed similar phenolics content. Nevertheless, *L. sativus* gave the highest flavonoids content and antioxidant activity in all the *in vitro* assays: DPPH scavenging activity, reducing power, β-carotene bleaching inhibition and TBARS formation inhibition (lowest EC_{50} values). This highlights flavonoids as the main phenolic compounds contributors for antioxidant activity. Other authors, such as Pastor-Cavada *et al.*[15], also reported a higher phenolic content and antioxidant activity for *Lathyrus* species (including *L. sativus*; 4.3 mg g^{-1}), when compared with commercial legumes such as *C. arietinum*. Nonetheless, according to Kanatt *et al.*[30] aqueous extracts of the latter species revealed excellent antioxidant activity, measured by DPPH and superoxide radical scavenging activity, β-carotene bleaching assay, reducing power and TBARS.

Soaking and specially cooking procedures increased phenolics and flavonoids content and, concomitantly, antioxidant activity measured by all the assays. This could be related to induced changes in phenolic compounds extractability due to the disruption of the plant cell wall, thus bound polyphenolic and flavonoid compounds may be released more easily relative to those of raw materials.[31] Otherwise, Starzynska-Janiszewska *et al.*[7] described a decrease in phenolics content from raw to cooked *L. sativus* samples (3.05 to 1.37 mg g^{-1}), and Nithiyanantham et al.[32] a decrease in antioxidant activity. These authors attributed the changes to several factors such as oxidative reaction, leaching of water-soluble antioxidant compositions, formation or breakdown of antioxidant compositions, and solid losses during processes.

Conclusion

L. sativus was the species with higher carbohydrates, proteins, ash, SFA and PUFA content, and with lower fat and energy value. Furthermore, it also showed the highest flavonoids concentration and antioxidant activity. *C. arietinum* gave the higher concentration of sugars, organic acids and tocopherols.

Soaking process did not affect significantly macronutrients, but cooking decreased protein, ash, sugars and organic acids, and increased carbohydrates, fat, tocopherols, bioactive compounds and antioxidant activity. No differences were obtained for fatty acids composition.

Finally, the present study highlights the nutritional value and bioactive properties of *C. arietinum* and *L. sativus* pulses, and valorises these traditional foods, referring to the interest of their inclusion in modern diets.

Acknowledgements

The authors are grateful to strategic project PEst-OE/AGR/UI0690/2011 (FCT, Portugal) for financial support to CIMO. Lillian Barros research contract from Foundation for Science and Technology- FCT Portugal (Programa Compromisso para a Ciência 2008).

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Table 1. Macronutrients composition and energetic value of Portuguese varieties of *C. arietinum* and *L. sativus* seeds (mean ± SD; n=3).

Energy (kcal kg^{-1} dw)	4081.50 ± 2.60^{c}	4171.82 ± 10.11^{b}	4261.92 ± 0.24^{a} *Cicer arietinum*		3883.65 ± 1.65^{b}	3896.72 ± 5.09^{b}	3957.67 ± 8.94^{a} *Lathyrus sativus*
		Raw	Soaked	Cooked	Raw	Soaked	Cooked
Ash (g kg^{-1} dw)		33.50 ± 0.14^{a}	32.65 ± 0.92^{a}	24.75 ± 2.05^{b}	35.80 ± 0.57^{a}	36.05 ± 0.21^{a}	29.15 ± 2.19^{b}
Proteins (g kg^{-1} dw)		283.27 ± 3.06^{a}	262.66 ± 19.41^{a}	198.68 ± 1.15^{b}	316.58 ± 12.83^{a}	289.47 ± 8.94^{a}	256.98 ± 15.76^{b}
Fat (g kg^{-1} dw)		43.10 ± 0.85^{b}	61.50 ± 2.12^{a}	72.20 ± 1.70^{a}	5.35 ± 0.92^{c}	8.20 ± 1.27^{b}	14.85 ± 0.78^{a}
Carbohydrates (g kg^{-1} dw)		640.69 ± 2.78^{b}	644.17 ± 19.02^{b}	704.35 ± 3.38^{a}	642.25 ± 12.32^{b}	666.26 ± 8.61^{b}	698.98 ± 16.33^{a}

dw- dry weight. Means in rows and for each pulses, followed by different letters differed significantly (p<0.05).

Table 2. Fatty acids composition of Portuguese varieties of *C. arietinum* and *L. sativus* seeds (mean ± SD; n=3).

	Cicer arietinum			*Lathyrus sativus*		
	Raw	Soaked	Cooked	Raw	Soaked	Cooked
C6:0	0.01 ± 0.00	0.01 ±0.00	tr	0.03 ± 0.01	0.02 ± 0.00	0.04 ± 0.00
C8:0	0.01 ± 0.00	0.01 ± 0.00	tr	0.06 ± 0.01	0.03 ± 0.01	0.04 ± 0.01
C10:0	0.01 ± 0.00	tr	tr	0.05 ± 0.01	0.03 ± 0.00	0.06 ± 0.00
C12:0	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.07 ± 0.00	0.06 ± 0.01	0.07 ± 0.00
C14:0	0.16 ± 0.01	0.15 ± 0.01	0.13 ± 0.00	0.52 ± 0.01	0.52 ± 0.02	0.43 ± 0.43
C14:1	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.05 ± 0.01	0.06 ± 0.00	0.03 ± 0.00
C15:0	0.06 ± 0.00	0.06 ± 0.00	0.07 ± 0.00	0.24 ± 0.02	0.24 ± 0.01	0.23 ± 0.23
C16:0	9.57 ± 0.56	9.12 ± 0.10	9.52 ± 0.01	8.84 ± 0.21	8.21 ± 0.27	8.53 ± 0.06
C16:1	0.27 ± 0.00	0.27 ± 0.01	0.25 ± 0.00	0.25 ± 0.01	0.25 ± 0.00	0.18 ± 0.00
C17:0	0.09 ± 0.01	0.08 ± 0.00	0.09 ± 0.00	0.35 ± 0.02	0.29 ± 0.02	0.39 ± 0.01
C18:0	1.33 ± 0.02	1.22 ± 0.09	1.28 ± 0.01	4.62 ± 0.10	4.73 ± 0.01	5.70 ± 0.07
C18:1n9	24.45 ± 0.81	23.41 ± 0.74	23.95 ± 0.02	16.75 ± 0.12	16.03 ± 0.17	16.14 ± 0.06
C18:2n6	58.91 ± 0.32	60.49 ± 0.79	59.63 ± 0.00	54.50 ± 0.43	56.81 ± 0.23	56.61 ± 0.16
C18:3n3	3.33 ± 0.02	3.48 ± 0.01	3.33 ± 0.00	9.32 ± 0.04	9.25 ± 0.03	9.01 ± 0.00
C20:0	0.51 ± 0.01	0.50 ± 0.00	0.47 ± 0.01	0.94 ± 0.06	0.91 ±	0.72 ± 0.02

					0.03	
C20:1	0.42 ± 0.01	0.40 ± 0.01	0.39 ± 0.02	0.58 ± 0.07	0.36 ± 0.02	0.36 ± 0.01
C20:2	0.09 ± 0.01	0.07 ± 0.00	0.10 ± 0.00	0.58 ± 0.05	0.17 ± 0.00	0.15 ± 0.02
C20:3n3+C21:0	0.05 ± 0.00	0.06 ± 0.00	0.06 ± 0.00	0.20 ± 0.02	0.19 ± 0.00	0.12 ± 0.00
C20:5n3	0.03 ± 0.01	0.03 ± 0.00	0.03 ± 0.00	tr	0.06 ± 0.00	0.02 ± 0.00
C22:0	0.33 ± 0.01	0.31 ± 0.00	0.30 ± 0.00	0.59 ± 0.00	0.47 ± 0.03	0.33 ± 0.02
C22:1n9	0.02 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.25 ± 0.01	0.25 ± 0.00	0.21 ± 0.00
C23:0	0.09 ± 0.01	0.09 ± 0.02	0.12 ± 0.01	0.29 ± 0.02	0.23 ± 0.01	0.15 ± 0.01
C24:0	0.21 ± 0.00	0.21 ± 0.02	0.23 ± 0.00	0.70 ± 0.02	0.54 ± 0.05	0.39 ± 0.02
C24:1	0.02 ± 0.00	0.02 ± 0.00	0.01 ± 0.00	0.23 ± 0.05	0.28 ± 0.07	0.10 ± 0.02
SFA (percentage)	12.41 ± 0.54^{a}	11.77 ± 0.04^{b}	12.23 ± 0.00^{a}	17.29 ± 0.23^{a}	16.28 ± 0.42^{c}	17.07 ± 0.23^{b}
MUFA (percentage)	25.18 ± 0.81^{a}	24.10 ± 0.76^{a}	24.62 ± 0.00^{a}	18.11 ± 0.11^{a}	17.23 ± 0.22^{b}	17.02 ± 0.09^{c}
PUFA (percentage)	62.41 ± 0.27^{b}	64.13 ± 0.80^{a}	63.14 ± 0.00^{b}	64.60 ± 0.12^{b}	66.49 ± 0.21^{a}	65.91 ± 0.14^{ab}

Caproic acid (C6:0); Caprylic acid (C8:0); Capric acid (C10:0); Lauric acid (C12:0); Myristic acid (C14:0); Myristoleic acid (C14:1); Pentadecanoic acid (C15:0); Palmitic acid (C16:0); Palmitoleic acid (C16:1); Heptadecanoic acid (C17:0); Stearic acid (C18:0); Oleic acid (C18:1n9c); Linoleic acid (C18:2n6c); α-Linolenic acid (C18:3n3); Arachidic acid (C20:0); Eicosenoic acid (C20:1c); *cis*-11,14-Eicosadienoic acid (C20:2c); *cis*-11, 14, 17-Eicosatrienoic acid and Heneicosanoic acid (C20:3n3 + C21:0); Eicosapentaenoic acid (C20:5n3); Behenic acid (C22:0); Erucic acid (C22:1n9); Tricosanoic acid (C23:0); Lignoceric acid (C24:0); Nervonic acid (C24:1); tr- traces. SFA- Saturated fatty aids; MUFA- Monounsaturated fatty acids; PUFA- Polyunsaturated fatty acids. Means of SFA, MUFA and PUFA in rows and for each pulses, followed by different letters differed significantly (p<0.05).

Table 3. Sugars, organic acids and tocopherols composition of Portuguese varieties of *C. arietinum* and *L. sativus* seeds (mean ± SD; n=3).

	Cicer arietinum			*Lathyrus sativus*		
	Raw	Soaked	Cooked	Raw	Soaked	Cooked
Fructose	8.39 ± 0.55^{a}	7.07 ± 0.51^{ab}	5.72 ± 0.64^{b}	nd	nd	nd
Sucrose	32.45 ± 0.74^{a}	30.30 ± 0.01^{b}	20.19 ± 0.32^{c}	25.33 ± 0.25^{a}	24.12 ± 0.74^{a}	21.22 ± 0.14^{b}
Trehalose	4.58 ± 0.25^{a}	4.43 ± 0.30^{a}	2.71 ± 0.11^{b}	nd	nd	nd
Raffinose	9.31 ± 0.52^{a}	7.62 ± 0.18^{b}	5.45 ± 0.69^{c}	nd	nd	nd
Stachyose	22.86 ± 0.27^{a}	22.53 ± 0.19^{a}	18.45 ± 0.22^{b}	nd	nd	nd
Verbascose	13.37 ± 0.22^{a}	12.17 ± 0.1^{b}	9.65 ± 0.05^{c}	nd	nd	nd
Total Sugars (g kg^{-1}, dw)	90.96 ± 0.03^{a}	84.12 ± 1.18^{b}	62.16 ± 0.72^{c}	25.33 ± 0.25^{a}	24.12 ± 0.74^{a}	21.22 ± 0.14^{b}
Oxalic acid	0.07 ± 0.01^{a}	0.07 ± 0.01^{a}	0.07 ± 0.01^{a}	4.77 ± 0.17^{a}	4.91 ± 0.11^{a}	4.88 ± 0.07^{a}
Malic acid	66.05 ± 0.64^{a}	50.13 ± 0.61^{b}	38.98 ± 1.46^{c}	19.28 ± 0.20^{a}	19.62 ± 1.47^{a}	14.66 ± 0.52^{b}
Citric acid	35.81 ± 0.49^{a}	23.60 ± 0.61^{c}	25.82 ± 0.40^{bc}	36.90 ± 1.48^{a}	23.88 ± 0.79^{b}	23.23 ± 0.05^{b}
Sum of organic acids (g kg^{-1}, dw)	101.93 ± 1.12^{a}	73.80 ± 0.62^{b}	64.87 ± 1.07^{c}	60.95 ± 1.44^{a}	48.41 ± 0.78^{b}	42.77 ± 0.64^{c}
α-tocopherol	23.63 ± 1.63^{c}	37.20 ± 0.20^{b}	44.16 ± 1.95^{a}	1.01 ± 0.01^{c}	1.54 ± 0.06^{b}	1.87 ± 0.17^{a}
β-tocopherol	0.72 ± 0.02^{c}	1.04 ± 0.05^{b}	1.34 ± 0.07^{a}	nd	nd	nd
γ-tocopherol	85.17 ± 5.34^{d}	134.50 ± 2.50^{b}	164.36 ± 3.52^{a}	62.32 ± 0.40^{c}	74.45 ± 4.56^{b}	101.53 ± 7.09^{a}
δ-tocopherol	7.56 ± 0.57^{c}	10.02 ± 0.11^{b}	12.09 ± 0.39^{a}	nd	nd	nd
Total tocopherols (mg kg^{-1}, dw)	117.08 ± 7.55^{c}	182.76 ± 2.24^{b}	221.95 ± 5.16^{a}	63.33 ± 0.38^{c}	75.99 ± 4.63^{b}	103.40 ± 7.26^{a}

nd- not detected; dw- dry weight. Means in rows and for each pulses, followed by different letters differed significantly (p<0.05).

Table 4. Non-nutrients composition and antioxidant properties of Portuguese varieties of *C. arietinum* and *L. sativus* seeds (mean ± SD; n=3).

	Cicer arietinum			*Lathyrus sativus*		
	Raw	Soaked	Cooked	Raw	Soaked	Cooked
Phenolics (mg GAE g^{-1} extract)	3.22 ± 0.06^{c}	5.25 ± 0.09^{b}	5.47 ± 0.05^{a}	3.15 ± 0.44^{c}	5.06 ± 0.29^{b}	5.59 ± 0.18^{a}
Flavonoids (mg CE g^{-1} extract)	1.56 ± 0.00^{c}	2.35 ± 0.00^{b}	2.71 ± 0.00^{a}	3.12 ± 0.00^{c}	4.29 ± 0.01^{b}	5.01 ± 0.01^{a}
DPPH scavenging activity (mg mL^{-1})	89.34 ± 0.85^{a}	52.04 ± 1.91^{b}	32.32 ± 1.25^{c}	37.97 ± 1.34^{a}	18.95 ± 0.64^{b}	15.23 ± 0.48^{c}
Reducing power (mg mL^{-1})	12.22 ± 0.03^{a}	11.43 ± 0.23^{b}	4.36 ± 0.16^{c}	3.99 ± 0.11^{a}	3.94 ± 0.06^{a}	3.26 ± 0.11^{b}
β-carotene bleaching inhibition (mg mL^{-1})	1.53 ± 0.13^{a}	1.03 ± 0.04^{b}	0.76 ± 0.02^{c}	1.04 ± 0.06^{a}	0.92 ± 0.16^{ab}	0.82 ± 0.08^{b}
TBARS inhibition (mg mL^{-1})	7.36 ± 0.11^{a}	5.71 ± 0.40^{b}	2.13 ± 0.56^{c}	4.53 ± 0.28^{a}	3.47 ± 0.25^{b}	1.62 ± 0.09^{c}

The antioxidant activity was expressed as EC_{50} values, what means that higher values correspond to lower reducing power or antioxidant potential. GAE and CE mean gallic acid and catechin equivalents, respectively. EC_{50}: Extract concentration corresponding to 50 % of antioxidant activity or 0.5 of absorbance for the reducing power assay. Trolox EC_{50} values: 30 μg mL^{-1} (reducing power), 43 μg mL^{-1} (DPPH scavenging activity), 3 μg mL^{-1} (β-carotene bleaching inhibition) and 4 μg mL^{-1} (TBARS inhibition). Means in rows and for each pulses, followed by different letters differed significantly ($p<0.05$).