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Nutritional values and radical scavenging capacities of grass pea (*Lathyrus sativus* L.) seeds in Valle Agricola district, Italy

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Abstract

Lathyrus sativus L., commonly known as grass pea, is an annual plant widely grown as a pulse crop and its dried seeds are harvested and consumed as a human food since ancient times. This plant is also commonly grown for animal feed and as forage. In the Mediterranean marginal areas, several grass pea germplasm *of Lathyrus sativus* L. are present and, among them the edible seeds of plants grown in Valle Agricola, a little town near Caserta (Italy), are well known for the local cuisine. Since there are no nutritional data available on the *Lathyrus sativus* grown in Valle Agricola, we have investigated nutritional values and metabolic profile of these seeds. Our results show that these seeds contain high levels of proteins (25.6±0.20 g/100 g) and essential amino acids (7.92 g/100 g). Different unsaturated fatty acids contribute to the total lipids amount (1.67±0.18 g/100 g); among them, the essential PUFA α-linolenic, linoleic and γ-linolenic acids are the most abundant. Ascorbic acid (13.50±0.30 mg/100 g) and glutathione (15.90±0.10 mg/100 g) are also present and, the folic acid content (206.70±8.30 μg/100 g) represents 50% of the vitamin RDA (Recommended Dietary Allowance). Total phenolic content (174.91±8.39/100 g), as well as the radical scavenging activity *vs*. DPPH radical and ABTS radical cation, have been estimated. The content of neurotoxin β-ODAP (16.2±0.5 g/Kg), commonly present in seeds of all examined grass pea genotypes and responsible of lathyrism paralysing disease, is quite high.

Keywords: Amino acids, Food, Grass pea, Nutritional values, Radical scavenging activity, Traditional foods.
Abbreviation: ABTS, 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonate); β-ODAP, β-diamino-propionic acid; DPPH, 2,2-diphenyl-1-picrylhydrazyl; *nor*-Leu, *nor*-leucine; RDA, Recommended Dietary Allowance; RP-HPLC, Reverse Phase High-Pressure Liquid Chromatography. For the amino acids the standard three-letter code has been used.

Introduction

In response to an ever increasing global demand for food and feed resources and to need to diversify modern cropping systems (Makoi and Ndakidemi, 2011), the legume genus Lathyrus is receiving increased attention by scientists. *Lathyrus sativus* L., common name grass pea (*Cicerchia* in Italian), is an annual pulse crop belonging to the *Fabaceae* family and *Vicieae* tribe (Biswas and Biswas, 1997). Grass pea plants show abiotic and biotic stress resistance. The plant is able to resist both drought and flooding, to adapt to a wide range of soil types, including the marginal ones, to grow in cool climates and at high altitudes. Compared to other legumes, it is also resistant to many insect pests (Tiwari and Campbell, 1996a; Tiwari and Campbell, 1996b; Berger et al., 1999; Sillero et al., 2005). Nowadays, grass pea is grown for stock-feed and human consumption in Asia (Bangladesh, China, India, Nepal and Pakistan) (Campbell, 1997), in the Middle East (Iraq, Iran, Afghanistan, Syria and Lebanon) (Campbell, 1997), in Northern Africa (Ethiopia, Egypt, Morocco, Algeria and Libya) (Girma et al., 2011) and in Southern Europe (France, Spain and Italy) (Campbell et al., 1994; Piergiovanni et al., 2011). Great taste and nutritional value of the seeds together with few growing requirements have gained the interest of the scientist community. In fact, these seeds have a good protein content (relatively rich in lysine) and a high level of polyunsaturated fatty acids (Chinnasamy et al., 2005). Nevertheless, in common with other grain legumes, grass pea seeds contain a variety of anti-nutritional factors (ANFs). In particular, β-diamino-propionic acid (β-ODAP), neurotoxic secondary metabolite, is a non-protein amino acid which causes neurolathyrism; this pathology appears when this molecule is ingested in large quantities over a three-to-four months period (Spencer et al., 1986; Enneking, 2011). The cooking procedures reduce the levels of the proteinaceous ANFs and of β-ODAP as well (Enneking, 2011). In Italy grass pea seeds have been part of the local diets for centuries up to post-war period; this crop was grown in Central and Southern Apennines (Toscana, Abruzzo, Puglia), where only this species of legume has been able to provide an acceptable food source for local farmers, thanks its hardiness. With globalization of markets and

approval of agricultural production, cultivation of grass pea has been almost completely abandoned over the past 50 years, while the production of more common species such as beans, lentils and chickpeas has prevailed. Nowadays grass peas are grown, using essentially traditional techniques, in marginal and remote areas characterized by specific climatic conditions. The renewed interest in grass pea cultivation is justified by the attempt to recover the crops belonging to popular tradition with the awareness that the maintenance of biological diversity is the key to any future strategy for sustainable production or for agricultural mechanization (Lak and Almassi, 2011). While many studies on the genetic characterization of Italian *L. sativus* exist (Granati et al., 2003; Hammer et al., 1992; Lioi et al., 2011; Tavoletti and Iommarini, 2007), only little information on nutritional values of *L. sativus* seeds is available; the only exception in this contest is represented by a recent study on the "cicerchie" of Campodimele (Latina, Italy) (Canini, 2004). Fairly consistent data on nutritional values of *L. sativus* seeds cultivated in Poland are instead available in literature (Lisiewska et al., 2003; Milczak et al., 2001). Availability of nutritional data is important both to understand the possible beneficial effects of *L. sativus* seeds consumption on human health and to support their possible production and marketing. In this framework, the present investigation is aimed to establish the nutritional properties of *L. sativus* seeds cultivated in the mountain community of Valle Agricola (Caserta, Southern Italy; 41°25'33"60 N, 14°15'21"24 E), in order to encourage their use as a local product. In this respect the total content of proteins, lipids, and sugars have been determined; quali-quantitative analyses of fatty acids, total and free amino acids have been also carried out. The content of ascorbic and folic acid, as well as of glutathione, was determined. The estimation of total phenols was performed by Folin Ciocalteau method. The antioxidant activity was also evaluated by measuring the ability of *L. sativus* seed extracts to scavenge the DPPH radical and the ABTS radical cation. Characterising studies on the nutritional qualities of these seeds obtained from Valle Agricola grass pea cultivation could stimulate their farming and sales.

Results

Nutritional values

Nutritional values of *L. sativus* seeds from Valle Agricola are reported in Table 1 and compared with data reported for Campodimele (Italy) and Poland (cv Derek) grass pea. Our results showed that nutritional values of Valle Agricola *L. sativus* seeds are similar to those of *L. sativus* seeds harvested in Campodimele, while there are some differences with polish cultivar. The protein content of Valle Agricola grass pea seeds (25.6±0.20 g/100 g) was slightly lower than that of the polish cultivar while lipid (1.67±0.18 g/100 g) and carbohydrate (72.91±2.95 g/100 g) levels are little higher.
Valle Agricola grass pea seeds had an important folic acid content (206.7±8.30 μg/100 g; Table 2). The content of vitamin B-complex is quite interesting and significantly higher than the content reported for other legumes products such as dried chickpeas flour (180 μg/100 g) and dried beans (130 μg/100 g). In *L. sativus* seeds from Valle Agricola the folic acid content represents the 50% of RDA (Recommended Dietary Allowance) uptake for adults (Barr, 2009). Ascorbic acid content (13.5 ± 0.30 mg/100 g) was lower than that of polish cultivar [268 mg/100 g; (Lisiewska et al., 2003)] but similar to other legumes (Jonathan Nugent and O'Connor, 2008). *L. sativus* seeds from Valle Agricola are also characterized by a high content of glutathione (15.9±0.10 mg/100 g) (Table 2) and by the absence of pentoses (arabinose, ribose and xylose) and hexoses (fructose, fucose, galactose, mannose and glucose).

Amino acid content

The contents of free and total (free plus protein-derived amino acids) amino acids are reported in Table 3 and compared with those available for polish *L. sativus* seeds (cv Derek). In our samples, two essential amino acids, leucine (Leu) and lysine (Lys) were the most abundant (1.79±0.096 and 1.76±0.111 g/100 g, respectively) when determined as total amino acids; among the non-essential amino acids, Glx (glutamic acid plus glutamine) and Asx (aspartic acid plus asparagine) were the most representative (9.53±0.636 and 3.35±0.293 g/100 g, respectively). Sulphur amino acids (cysteine and methionine, 0.26±0.012 and 0.19±0.003 g/100 g, respectively) resulted twice the amount than those found in polish grass pea. Total free amino acid content of Valle Agricola *L. sativus* seeds was 112.0 mg per 100 g. Among the essential amino acids, the most abundant free amino acids were valine (Val) and methionine (Met) (6.62±0.180 and 4.56±0.019 mg/100 g, respectively). Free asparagine (Asn), arginine (Arg) and glutamic acid (Glu) (54.26±0.830, 12.26± 0.660 and 5.64±0.110 mg/100 g, respectively) were the main non-essential amino acids. This analysis also evidenced the presence of non-protein amino acids whose total amount was 10.17 mg/100 g; in particular, α-aminoadipic acid (AAAA) and sarcosine (Sarc) were the most abundant (3.54±0.07 and 3.26±0.13 mg/100 g, respectively) whereas the remaining non-proteic amino acids did not exceed 3.5 mg/100 g of product.

Fatty acid composition analysis

The fatty acid composition showed that saturated acid content was higher (369.32 g/100 g; 53.69%) than that of unsaturated acids (322.44 g/100 g; 46.61%; Table 4). The most abundant saturated acids were stearic (C18:0) and margaric (C17:0) acids, representing 38.12% and 15.26% respectively, of total fatty acids. The presence of odd-numbered fatty acids as margaric acid has already been detected in some *Lathyrus* taxa belonging to different sections from Turkey (Bağci and Şahın, 2004); these authors observed that the total content of fatty acid as well as the composition of seed lipids can serve as taxonomic markers in higher plants. Oleic acid (n-9; 18:1) was the main component of the unsaturated fatty acid fraction (139.7 mg/100 g). Linoleic acid (n-6; C18:2), α-linolenic acid (n-3; C18:3), and γ-linolenic acid (n-6; C18:3) were the main unsaturated fatty acid components (Table 4). In addition, Valle Agricola grass pea seeds showed slightly higher amounts of saturated fatty acids than unsaturated fatty acids; this finding confirms data reported for the polish grass pea seeds (Grela et al., 1999) and others legumes (Grela and Günter, 1995).

Total phenol content

The content of total phenols evidenced a relative abundance of phenolic phytochemicals in Valle Agricola *L. sativum*

Table 1. Nutritional data of Valle Agricola, Campodimele and Poland (cv Derek) grass pea seeds. Values are means (±SD) of triplicate analyses (n = 3) and are expressed on weight basis.

L. sativus samples	Moisture (%)	Ash (%)	Proteins (g/100g)	Lipids (g/100g)	Carbohydrates (g/100g)
Valle Agricola	9.10	3.00±0.10	25.60±0.20	1.67±0.18	72.91±2.95
Campodimele [a]	10.44	2.92	23.93	1.50	n.r.
Poland, cv. Derek	n.r.	3.30	29.00	1.30	65.50

n.r., not reported; a, taken from Canini, 2004.

Fig 1. The arrow indicates Valle Agricola grass pea seeds sampling area.

seeds. In fact, all the tested samples yielded average value of 175.0±8.39 mg/100 g, expressed as gallic acid equivalents (GAE; see Methods). The result was in agreement with those obtained for seeds grown in Campodimele (74.5 to 263.0 mg/100 g GAE) (Canini, 2004). Our data resulted lower than those reported for grass pea seeds grown in south Spain (430 mg/100 g) (Pastor-Cavada et al., 2009).

Radical scavenging activity

Extracts of grass pea flour were investigated for the radical scavenging capacity by using ABTS and DPPH assays; this investigation is characterized by a simple and rapid execution and good reproducibility, and consist of specific redox reactions in which the radical acts as an oxidant and probe (Fig. 2). Extracts of grass pea flour exercised a weak conversion of the DPPH radical, estimable in a reduction of 36.3% at the highest tested dose. Furthermore, the investigated flour extracts showed a moderate reducing capability of ABTS radical cation. The activity became remarkable at 250 μg/mL as the ABTS radical cation reduction was 47.1%.

Content of β-ODAP

The β-ODAP amount was 16.2±0.5 g/Kg, evidencing that the analysed Valle Agricola *L. sativus* seeds do not belong to low toxic varieties in which the level of β-ODAP is below 1 g/Kg (Castell et al., 1994; Campbell, 1997). However, as previously reported, after boiling, *L. sativum* seeds did not shown any β-ODAP content; this result suggests the efficiently of this process in removing the neurotoxin.

Discussion

L. sativus produces seeds used in the local diets of Mediterranean countries for millennia. In this study we have characterized for the first time the nutritional values of seeds obtained by grass pea germplasm grown in Valle Agricola. The results confirm that Valle Agricola grass pea is an important source of many essential nutrients. Its high value of total proteins (25.60±0.20 g/100 g) was similar to that reported for Campodimele variety [23.93 g/100 g; (Canini, 2004)] and slightly lower than that of cv Derek from Poland [29.0 g/100 g (Milczak et al., 2001)]. The total amino acid amount of Valle Agricola grass pea seeds (29.07 g/100 g) is higher than that of the polish cultivar (9.15 g/100 g) and this incongruity is likely due to the different methodologies used (Csapò et al., 2008). Total amount of Asparagine was the main free amino acid in line with its role as *N*-mobilizer in plant species (Brouquisse et al., 1998). The lipid content was similar to that reported for Campodimele variety. In this work a qualitative analysis of the fatty acid composition showed that among the unsaturated fatty acids, the amount of the essential n-6 linoleic (LA, 18:2 n-6), n-3 α-linolenic (α-LNA, 18:3 n-3) and linoleic (LA; 18:2) acids were higher in Valle Agricola grass pea seeds. Total phenol content was similar to that of grass pea grown in Campodimele (on average 168.5 mg/100 g). The presence of phenol phytochemicals justifies the radical scavenging activity observed and addresses the extension of the seeds cultivation as new source of natural antioxidants besides containing high quality proteins for human or animal nutrition. The radical scavenging activity investigation by ABTS and DPPH methods put its basis on previous studies (Starzyńska-Janiszewska et al., 2008) in which the authors, studying the antiradical and total antioxidant effects from raw (prepared for inoculation), fermented and cooked seeds of grass pea (*L. sativus* Krab and Derek cultivars), observed that the approximate activity of extracts from both grass pea cultivars against ABTS radical cation was about 4-fold higher than the activity measured with the DPPH radical. Antioxidant content of Valle Agricola grass pea seeds resulted also from the presence of glutathione and ascorbic acid, higher than that reported for other legumes. In particular, reduced glutathione (GSH), a tripeptide (γ-glutamyl-cysteinylglycine), is the major free thiol in most living cells and is involved in many biological processes such as detoxification of xenobiotics, removal of hydroperoxides, and maintenance of the protein sulfydryls. It

Table 2. Vitamin and other metabolites content of Valle Agricola grass pea (100 g). Values are means (±SD) of triplicate analyses (n = 3) and are expressed on weight basis.

Component	Content
Folic acid	206.70±8.30 μg
Vitamin C	13.50±0.30 mg
Glutathione	15.90±0.10 mg
Phenolic compounds	174.91±8.39 mg

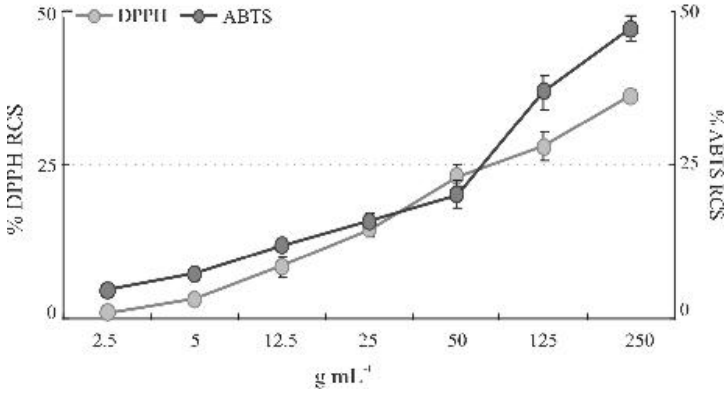

Fig 2. Radical Scavenging Capacity (RSC, %) of grass pea extracts on DPPH$^{\bullet}$ and ABTS$^{\bullet}$ cation. Values, reported as percentage *vs*. a blank, are the mean ± SD of three measurements (n = 3).

is the key antioxidant in animal tissues. Folic acid plays an important role in the nucleic acid and protein synthesis and in erythropoiesis. It is particularly important for tissues that undergo proliferation and differentiation. For these reasons it could be essential in preventing birth defects. The level of β-ODAP in our samples was 16.2 g/ Kg. In the literature the content of β-ODAP in *L. sativus* seeds evaluated by other authors is quite variable [between 0.22 and 7.2 g/Kg; (Castell et al., 1994)].

Materials and methods

Chemicals and reagents

Nonadecanoic acid, *nor*-leucine (*nor*-Leu), Folin-Ciocalteau reagent, gallic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH·), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), *o*-phthalaldehyde reagent (OPA), DL-2,3-diamino propionic acid (DAP), phenol red, hydrogen peroxide, peroxidase from horseradish, solvents and salts were obtained from Sigma-Aldrich S.r.l. (Milan, Italy). Fatty acid methyl esters (Supelco™ 37 component FAME mix) were obtained from Supelco (Park-Bellefonte, PA, USA). Chemicals and solvents for the Kjeldahl method and for the automated amino acid analysis were provided by Carlo Erba Reagents (Milan, Italy) and Biochrom (Cambridge, U.K.), respectively.

Plant material and sampling

L. sativus seeds were grown under typical conditions of soil, irrigation, and illumination in Valle Agricola, 71 km Northwest from Caserta, Italy (geographical coordinates: 41°25'33"60 N, 14°15'21"24 E; Fig. 1). In particular, *L. sativus* seeds were harvested from reference field. From field, about 500 mature seeds (~110 g), with an approximate diameter of 0.60 cm, were manually and randomly picked across the fields in July (Summer) of 2009; dried for 120 days at room temperature as custom of the place. Then, samples were selected to eliminate damaged and poor quality units and to obtain uniformity. For the analysis, samples were cleaned with MilliQ water, drained, and gently dried with a paper towel. Seeds were then powdered with the Cyclone Sample Mill instrument (PBI International, Milan, Italy), until flour of homogeneous size was obtained. Flour was transferred into 50 mL polypropylene bottles (Falcon, Becton Drive, Franklin Lakes, NJ, USA), covered with silver paper and stored at -80 °C until use. All measurements were performed by triplicate.

Ash and moisture content

Ash content and moisture level were determined according to the AOAC official method (AOAC, 1997). Sample (2.0 g) was dried in a thermostatically controlled oven, at uniform temperature of 550 °C and 105 °C, respectively, until the constant weight was obtained.

Macronutrient content

Total protein content: Nitrogen concentration was obtained by the Kjeldahl method (AOAC, 1997) and total protein content was estimated using a nitrogen factor of 6.25. Samples (about 1.0 g) were analyzed using a Mineral Six digester and an Auto Disteam semi-automatic distilling unit (International PBI). *Total lipid content:* Aliquots (5.0 g) of frozen flour grass pea sample were lyophilized using an FTS-System Flex-Dry™ instrument (SP Scientific, Stone Ridge, NY USA). The materials obtained were extracted by the Soxhlet apparatus with $CHCl_3$ for 4 h and dried using a rotary evaporator to obtain the crude extracts which were weighed giving the amount of extracted fat. *Total carbohydrate content:* The carbohydrate content was obtained by subtracting the value of total ash, lipids and proteins from the total dry matter.

Amino acid composition

For the analysis of free amino acid composition, aliquots of about 200 mg of seed flour were precipitated with 80% cold ethanol (1.0 mL), in the presence of *nor*-Leu (50.0 nmol) as internal standard, homogenized with a teflon pestle and centrifuged at 14000*g*, at 4 °C. The supernatant was lyophilized, treated with 3% sulfosalicylic acid (500 μL) to precipitate any protein fraction still present, and centrifuged again (Di Maro et al., 2011). The supernatant was then analysed. For the analysis of total (free and protein) amino acids, about 10 mg of seed flour were hydrolysed with 0.5 ml of 6 N HCl containing 0.02% phenol and *nor*-Leu as internal standard at 110 °C for 20 h (Iriti et al., 2009). Following hydrolysis, HCl was removed under *vacuum* and samples resuspended in 0.5 mL of 0.2 M lithium citrate buffer (pH 2.2). Aliquots of both samples were directly analysed on a Biochrom 20 amino acid analyser (Biochrom, Cambridge, U.K.), equipped with a post-column ninhydrin derivatization system, adapting the procedure previously reported (Stein and Moore, 1963).

Table 3. Free and total amino acid composition of Valle Agricola and cv. Derek grass pea seeds. Protein amino acids are reported in bold. Values are means (±SD) of triplicate analyses (n = 3) and are expressed on weight basis.

	Valle Agricola		cv Derek
Amino acid[a]	Total amino acids [b] (g/100g)	Free amino acid content [(g/100g) 10^{-3}]	Total amino acids (g/100g)
essential amino acids			
His*	0.52±0.01	0.11±0.01	0.26
Ile	0.95±0.04	0.65±0.03	0.44
Leu	1.79±0.10	2.10±0.07	0.56
Lys	1.76±0.11	1.87±0.09	0.60
Met	0.19±0.01	4.56±0.02	0.09
Phe	0.75±0.03	1.49±0.03	0.43
Thr	0.83±0.04	0.43±0.010	0.41
Trp	n.d.	3.33 ±0.10	n.r.
Val	1.13±0.06	6.62±0.18	0.51
non essential amino acids			
1-mhis	n.d.	0.53±0.02	n.r.
AAAA	n.d.	3.54±0.07	n.r.
Ala	1.19±0.08	1.94±0.04	0.51
Arg	2.03±0.14	12.26±0.66	0.80
Asn	-	54.26±0.83	-
Asp	-	1.97±0.09	-
Asx	3.35±0.29	-	0.93
Car	n.d.	1.90±0.03	n.d.
Cys	0.26±0.01	nd	0.10
GABA	n.d.	0.94±0.03	n.r.
Gln	-	1.46±0.03	-
Glu	-	5.64±0.11	-
Glx	9.53±0.64	-	1.92
Gly	1.66±0.70	1.79±0.06	0.43
Pro	1.65±0.16	0.92±0.04	0.48
Sarc	n.d.	3.26±0.13	n.r.
Ser	0.98±0.06	0.29±0.01	0.37
Tyr	0.51±0.01	0.42±0.02	0.31
Total (g)	29.07	0.112	9.15

[a] free and protein amino acids. Three letter code has been used: 1-mhis: 1-methyl-L-histidine; AAAA: L-α-aminoadipic acid; Ala: L-alanine; Asn: L-asparagine; Arg: L-arginine; Asp: L-aspartic acid; Asx: L-asparagine plus L-aspartic acid; Car: L-carnosine; Cys: L-half cystine; GABA: γ-amino-n-butyric acid; Gln: L-glutamine; Glu: L-glutamic acid; GLx: L-glutamine plus Glu: L-glutamic acid; Gly: glycine; His: L-Histidine; Ile: L-isoleucine; Leu: L-leucine; Lys: L-lysine; Met: L-methionine; Phe: L-phenylalanine; Pro: L-proline; Sarc: L-sarcosine; Ser: L-serine; Thr: L-threonine; Trp: L-tryptophan; Tyr: L-tyrosine; Val: L-valine. [b] free plus protein-derived amino acids (see text). [c]free amino acids content for Derek and other cultivars is not previously published. n.d., not determined, n.r., not reported.

Table 4. Fatty acid composition before extraction from total lipids of Valle Agricola grass pea seeds. Values are means (±SD) of triplicate analyses (n = 3) and are expressed on weight basis.

Fatty acid		Fatty acid content (mg/100g)	Types of omega fatty acids
saturated			
lauric	C12:0	*traces*	-
tridecylic	C13:0	*traces*	-
pentadecylic	C15:0	*traces*	-
palmitic	C16:0	*traces*	-
margaric	C17:0	105.60±8.58	-
stearic	C18:0	263.72±26.87	-
unsaturated			
oleic	C18:1 *c*	139.69±4.52	n-9
linoleic	C18:2	85.59±6.78	n-6
α-linolenic	C18:3		n-3
and	+	90.22±9.19	
γ-linolenic	C18:3		n-6
erucic	C22:1	6.94±0.70	n-9
Total fatty acids		691.76	

Gas chromatographic analysis of fatty acid methyl esters

Fatty cid methyl esters content were performed by a gas chromatographic analysis as previously reported (Ferrara et al.,2011),using $CHCl_3$ crude extract (1.0 mg; see "Macronutrient content" section).

Free sugar (pentoses and hexoses) content

The free monosaccharide composition, pentoses (arabinose, ribose and xilose) and hexoses (fructose, galactose, mannose, glucose and fucose) was determined using a Bio-LC® (Dionex Corp., Sunnyvale, CA, USA) equipped with a CarboPac® PA10 column (2 × 250 mm, Dionex Milan, Italy) and a guard column Amino Trap™ (2 × 50 mm, Dionex Milan, Italy). Protocols employed were as suggested by the manufacturer (Technical Note 40 by Dionex Corp., Sunnyvale, CA, USA).

Folic acid and ascorbic acid content

Folic acid content was detected using the enzyme immunoassay for the quantitative determination of folic acid in food (cod. ELISA kit 010060; EuroKit Srl, Gorizia, Italy) as previously reported (Ferrara et al., 2011). Ascorbic acid content was determined using the HA 3850 kit (specific for this vitamin; HANNA Intruments, Ronchi di Villafranca, Padova, Italy) as previously reported (Ferrara et al., 2011). This titration method only determines ascorbic acid and not dehydroascrobic acid (DHA).

Glutathione determination

The glutathione determination was performed using the Glutathione Assay Kit (Sigma-Aldrich, S.r.l.). A standard glutathione curve and samples were determined by serial dilution as suggested by manufacturer's instructions.

Determination of total phenol content

In order to define total phenol content, aliquots (5.0 g) of frozen flour grass pea sample were lyophilized using an FTS-System Flex-Dry™ instrument (SP Scientific). Samples (1.0 g) were extracted with ethanol (70%, v/v; 3 × 100 mL) acidified to pH 2.0 with formic acid, after the samples were defatted with *n*-hexane (4 × 20 mL) and concentrated under vacuum to a final volume of 10 ml (Romani et al., 2006). The obtained ethanolic extracts were used for the determination of total phenol content which was determined according to the Folin-Ciocalteau procedure (Kähkönen et al., 1999). Total phenol content was expressed as gallic acid equivalents (GAE, mg/g of seed flour).

Determination of radical scavenging capacity

Aliquots (1.0 g) of frozen flour grass pea sample were lyophilized using an FTS-System Flex-Dry™ instrument (SP Scientific). Samples (1.0 g) were extracted with methanol. Then, extract was centrifuged (14000*g*, at 4 °C for 15 min). The clear supernatant was assayed after proper dilution.

DPPH$^{\bullet}$ method

Determination of 2,2-diphenyl-1-picrylhydrazyl radical ($DPPH^{\bullet}$) scavenging capacity was estimated by the Brand-Williams method with slight modification (Brand-Williams et al., 1995).The results are expressed as percentage of reduction of the initial DPPH adsorption by test samples.

ABTS radical cation method

Determination of 2,2'-azinobis 3-ethylbenzotiazolyn-6-sulfonic acid ($ABTS^{\cdot+}$) scavenging capacity was evaluated according to the Gallati method (Gallati, 1979). The radical scavenging capacity was expressed as a percentage increase of the initial absorption of the ABTS.

Determination of β-ODAP level

β-ODAP content was determined as reported (Granati et al., 2003). A standard curve was determined using DL-2,3-diamino propionic acid (DAP). In addition, in order to verify if the neurotoxin was released after boiling, seeds were soaked overnight and boiled until they softened.

Statistical analysis

Analyses were repeated three times for sample; mean and standard deviation (SD) of experimental values are reported, and the evaluation of the statistical significance of results was based on the Student's t test for paired data analysis, with a probability value of < 0.05 ($p < 0.05$) considered to be statistically significant.

Conclusions

Although the seeds of *L. sativus* have been consumed for centuries as a legume, the plant is not intensively cultivated in Italy. Recently, the interest in its cultivation has been increased, but still there are little information on the nutritional value of this legume. The data obtained from our analyses emphasized the Valle Agricola grass pea seeds high content of proteins, folic acid (50% of the RDA) and unsaturated fatty acids. The quali-quantitative analysis of free amino acids was reported for the first time. The elicited scavenging capability and the high total phenol content of Valle Agricola grass pea seeds let to consider it a nutritionally attractive resource. The wholesomeness of the legume is increased by the boiling process which favors the removal of the toxic β-ODAP molecule. Moreover, *L. sativus* is also interesting for the local ecosystems, because it fits well on slopes and in drought periods, reducing soil erosion and leading to the recovery of unproductive fields.

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