

NUTRITIONAL VALUES AND METABOLIC PROFILE WITH AND WITHOUT BOILED TREATMENT OF ‘GALLO MATESE’ BEANS (*PHASEOLUS VULGARIS* L.), A LANDRACE FROM SOUTHERN ITALY*

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ABSTRACT

Background. ‘Gallo Matese’ beans are known as a typical legume of Southern Italy and continue to be consumed as a traditional food preserving the diversity of this region. Nonetheless, no information about the nutritional values of this legume is available. The objective of the present investigation was to determine the nutritional value and metabolic profile of ‘Gallo Matese’ beans.

Results. ‘Gallo Matese’ beans contain high levels of proteins (22.64 g/100 g) and essential amino acids (8.3 g/100 g). Furthermore, different unsaturated fatty acids contribute to the total amount of lipids (0.97 g/100 g); among them, the essential PUFA α -linolenic (0.48 g/100 g) and linoleic (0.39 g/100 g) acids are the most abundant. The total phenol content was revealed and ABTS and ORAC-fluorescein methods were applied to determine the radical scavenging capabilities of the extract with and without boiled treatment. Finally, a decrease in trypsin and chymotrypsin inhibitory activities was estimated before and after the boiling procedure.

Conclusion. The data obtained show that ‘Gallo Matese’ beans are a functional food with healthy qualities. Overall, these results are useful to promote their cultivation and consumption, thus preserving Italian beans biodiversity due to consumer interest in choosing a healthy diet, such as the Mediterranean diet.

Keywords: amino acids, antioxidants, fatty acids, legumes, food quality

INTRODUCTION

The Fabaceae or Leguminosae, commonly known as the Legume family, are flowering plants intensively cultivated for the production of their grain seed, called pulse, which is a rich source of vegetable proteins, dietary fibers, carbohydrates, vitamins and dietary minerals (Mudryj et al., 2014). These seeds are important

not only in both economical and nutritional terms, but also for biological nitrogen fixation (Nulik et al., 2013).

The most popular seeds of Leguminosae are soya, pea, lentil and broad or common beans; whose consumption is significantly increased due to a vegetarian

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lifestyle, as legumes offer a healthy alternative to meat proteins (Nelson et al., 2016). In fact, although legumes are low in sulphur-containing amino acids, their usual combination with other sources such as cereals, roots and tubers, provides great protein quality (Iqbal et al., 2006). Furthermore, pulses are beneficial because of their low glycemic index (Jenkins et al., 2012) and their consumption has a positive effect on several other cardiovascular disease risk factors, such as blood pressure, platelet activity, and inflammation (Bazzano et al., 2001). In addition, many phytochemicals found in legumes possess antioxidant (Amarowicz and Pegg, 2008) and anti-carcinogenic (Mudryj et al., 2014) qualities.

Common bean seeds of *Phaseolus vulgaris* L., are the most consumed legume crops, second to soya beans in global legume seed cultivation (Nedumaran et al., 2015). The plant of *P. vulgaris* and therefore its seeds, known at least seven thousand years ago in the Americas, were probably introduced to Europe from Mesoamerica (Zeven, 1997). After its importation, the common bean spread over the European continent through specific agronomic characteristics such as: 1) cultivation in the warm season that does not tolerate long cold periods; 2) moderate rainfall, particular essential during and immediately after the flowering stage; 3) not sensitive to soil type, as long as it is reasonably fertile (Katungi et al., 2009). On the other hand, the consumption of common beans, along with maize, potato, pumpkin and pears from the Americas, was one of the causes of the demographic increase registered in Europe between the seventeenth and eighteenth centuries (Lee, 1982). The seeds of *P. vulgaris* were probably introduced to Italian territory from Spain and over time spread in an extensive manner (Piergiorganni and Lioi, 2010). The peculiar pedoclimatic characteristics of Italian territory, such as soil type, climatic condition or geographical isolation produced a myriad of landraces (Comes, 1910). This process, made easier because of selective pressure exerted by Italian farmers motivated by aesthetic and organoleptic preferences, led to the emergence of about five hundred species in Italy (Piergiorganni and Lioi, 2010). Nowadays, the nutritional and health benefits of common bean consumption in Italian and Mediterranean countries have made this legume one of the key foods in the Mediterranean diet (Widmer et al., 2015).

In particular, in the Campania region more than twenty different types of beans are known as traditional regional foods (Gazzetta_Ufficiale, 2016; Piergiorganni et al., 2015; Scarano et al., 2014) and some of them, such as 'Occhio nero di Oliveto Citra' (Zaccardelli et al., 2013) or 'Controne' beans (Del Piano et al., 2012; Mennella et al., 2003) are the subject of study.

Within this framework, our group aims to investigate the biochemical and nutritional properties of autochthonous beans of Campania region in order to promote their consumption at local and international levels, and has decided to characterize 'Gallo Matese' beans in the way already done for other typical cultivars (Dosi et al., 2013; Landi et al., 2015; Pacifico et al., 2014; Tamburino et al., 2012a). These dry legumes, produced in low quantities through family cultivation, are high-quality beans and are mainly sold in the local market. 'Gallo Matese' beans take their name from Gallo Matese, a small town located near Caserta in the Matese Mountain chain. They are a local ecotype with ocher-colored pods, a small size and white flowers. The plants, one meter high, with indeterminate growth, have a climbing habit and traditionally grow on local red wheat. The seeds are white and small with a hilar scar, thin skin, delicate flavour, high digestibility and a shape that visually resembles a kidney,

In this framework, macronutrient content as well as total and free amino acids and fatty acids were determined. Furthermore, the antioxidant capability of raw and boiled 'Gallo Matese' beans was estimated in terms of total phenol content, ORAC value and ABTS radical scavenging activity. Finally, due to common beans being known to contain anti-nutritional factors such as trypsin and chymotrypsin inhibitors, they were analysed with and without boiling.

MATERIAL AND METHODS

Chemicals and reagents

2,2'-azobis-(2-amidinopropane)-dihydrochloride (AAPH), *nor*-leucine (*nor*-Leu), fluorescein, Folin-Ciocalteu reagent, gallic acid, salts and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox[®]) were purchased from Sigma-Aldrich (Milan, Italy). Solvents were from Sigma-Aldrich. Chemicals and solvents for the Kjeldahl method were from Carlo Erba Reagents (Milan, Italy), whereas those for

automated amino acid analysis were provided from Biochrom (Cambridge, U.K.).

Material and sampling of 'Gallo Matese' bean seeds

P. vulgaris seeds were grown in typical soil and illumination conditions in Gallo Matese, ~70 km north-west of Caserta, Italy (geographical coordinates: 41°28'N 14°13'E). Bean seeds manually and randomly harvested across the reference field in 2016, and collected in two different territory of Gallo Matese, were then sun-dried for some weeks. Dried bean seeds were powdered with the Cyclone Sample Mill Instrument (PBI International, Milan, Italy), until flour of a homogeneous size was obtained. The material, hereafter "seed flour", was stored at a temperature of -20°C in plastic bags and in the dark. All measurements were performed in triplicate.

Ash and moisture content

The ash content and moisture level were determined according to the AOAC official method (AOAC, 1997).

Macronutrient content

Total raw protein (AOAC, 1997), lipid (by using Soxhlet apparatus using CHCl₃ as extracting solvent) and carbohydrate (FAO, 2003) content was determined as previously reported (Landi et al., 2017; Landi et al., 2015).

Amino acid composition

For the analysis of free amino acid composition, three aliquots of seed flour (~200 mg) were precipitated with 80% cold ethanol (1.0 mL), in the presence of *nor*-Leu (50.0 nmol) as an internal standard, homogenized with a teflon pestle and centrifuged at 14 000 ×g, at 4°C. The supernatants were lyophilized, treated with 3% sulfosalicylic acid (500 µL) to precipitate any protein fraction still present, and centrifuged again (Iriti et al., 2009). For the analysis of total (free and protein) amino acids, ~10 mg of seeds flour were hydrolysed with 0.5 mL of 6 N HCl containing 0.05% thioglycolic acid and *nor*-Leu as an internal standard at 110°C for 20 h and treated as previously reported (Ferrara et al., 2011). Aliquots of hydrolysed and non-hydrolysed samples were directly analysed by using a Biochrom30 amino acid analyser (Biochrom, Cambridge, U.K.).

Evaluation of the antioxidant capacity of 'Gallo Matese' beans

The total phenol content (TPC) and antioxidant capability of seed flour were measured on acetone/water extracts previously obtained through maceration. For this purpose three replicate seed flour samples (~200 mg) were extracted in 5 mL of acetone/water (80:20, v/v) for 3 h in a rotary shaker. After this period, the samples were centrifuged at 10 000 g for 30 min. The supernatant was filtered through a 0.22 µm PVDF filter (Anton et al., 2009). The extract was stored at -20°C until use. Furthermore, in order to evaluate the effect of boiling (the most commonly used cooking method for common beans) on phenol content and antioxidant capacity, three replicate samples (5.0 g each) of raw material were first boiled in water (50 mL, each). After 120 min, the cooked beans and the cooking water were freeze-dried. The samples obtained were extracted as described above. Folin-Ciocalteu, ABTS⁺ and ORAC assays were carried out by performing three replicate measurements for three samples (*n* = 3) of each extract. The activities recorded were compared to a blank.

Total phenol content determination

The total phenol amount of seed flour was determined according to the Folin-Ciocalteu procedure on aliquots of extracts in acetone/waters. The content of the TPC value of the samples was expressed as mg gallic acid equivalents (GAE) per 100 g of seed flour.

Determination of ABTS radical cation scavenging capacity

The ABTS⁺ solution scavenging capacity of seed flour was estimated as previously reported (Tamburino et al., 2012a). The results were expressed in terms of TEAC values (µmol Trolox[®] Equivalents per 100 g of seed flour).

Oxygen radical absorbance capacity assay (ORAC)

The antioxidant potential of seed flour was measured by Oxygen Radical Absorbance capacity (ORAC) assay as previously reported (Landi et al., 2015; Landi et al., 2017). The analysis was performed using 96-well microplates in which 25 µL of appropriately

diluted acetone/water extract from raw, cooked common beans with and without cooking water. The antioxidant activity (ORAC value) was calculated by using the Trolox[®] calibration curve. The ORAC value was expressed as Trolox[®] equivalents (μmol Trolox per 100 g of seed flour).

Determination of trypsin and chymotrypsin inhibitory activities

Three replicate samples of seed flour (1.0 g each) were extracted overnight under magnetic stirring at 4°C in 80 mM Tris•Cl, pH 7.8, containing 0.1 M CaCl₂ (1:4; w/v). The reaction mixtures were centrifuged at 24 000 g (Centrifuge Avant J-25, Beckman Coulter, CA, USA) at 4°C for 60 min. The supernatants were first filtered on Miracloth (pore size: 22–25 μm) and then freeze-dried.

The protein concentration was determined with the Bio-Rad Protein Assay kit following the manufacturer's instructions and using bovine serum albumin (BSA) as standard.

Trypsin and anti-trypsin activities, as well as chymotrypsin and anti-chymotrypsin activities, were determined following a procedure already described (Tamburino et al., 2012b), using TAME (*p*-toluenesulfonyl-L-arginine methyl ester) and BTEE (N-benzoyl-L-Tyrosine ethyl ester) as substrates, respectively. One inhibitory unit is defined as the amount of inhibitor that, yields a 30% decrease in the assayed enzymatic activity under assay conditions. An increasing concentration of seed protein extract was added to a fixed concentration of enzyme, which in our conditions corresponded to 0.05 absorbance/min monitoring for 5 min of the reaction. Residual enzyme activities are expressed as a percentage of the control samples (enzyme activity in the absence of protein extract). The IC₅₀ values of trypsin and α -chymotrypsin activities (i.e. the half maximal inhibitory concentration) of bean protein extract were calculated as previously reported (Tamburino et al., 2012b).

Gas chromatographic analysis of fatty acid methyl esters

The analysis of fatty acid methyl ester content was performed as previously reported (Ferrara et al., 2011). The fatty acid methyl esters were identified by comparing their retention times with those of standard

fatty acid methyl esters (Grain Fatty Acid Methyl Ester mix 47801, Supelco).

Free glucose content

To determine the glucose level, three aliquots of seeds flour (~30 mg) were extract as previously reported (Bailly et al., 2001). The dry extracts were dissolved in 100 μL of milliQ water. The samples (20 μL) were assayed according to the Glucose LR procedure kit, an enzymatic colorimetric method, following the manufacturer's instruction (Glucose LR, SGM Italia, Rome, Italy).

Statistical analysis

The analyses were repeated three times for each sample; the mean and standard deviation (SD) of experimental values were reported. Data analysis was carried out with Excel Office 2010 (Microsoft Corporation, Redmond, WA, USA). The IC₅₀ values were calculated based on inhibition curves by plotting the residual enzyme activities *versus* different concentrations of protein extract by fitting data with a non-linear regression analysis on a semi-logarithm scale by using the GraphPad Prism 5 software (GraphPad software Inc., California, USA). All measurements were performed in triplicate and the results are reported as mean \pm SD values. The Bonferroni post-test was used to determine significant differences between 'Gallo Matese' bean seeds collected at two different sites, named C1 and C2. The test was performed using a $P < 0.05$.

RESULTS AND DISCUSSION

Nutritional values

In order to promote 'Gallo Matese' beans, autochthonous beans of the Campania Region, a thorough investigation of their biochemical and nutritional properties was carried out following a workflow reported in Figure 1.

The nutritional values of dried bean seeds from Gallo Matese (hereafter 'Gallo Matese' beans) obtained by analysis of two different sites (named C1 and C2) and their average (\bar{x}) are reported in Table 1. On the other hand, since the statistical analysis did not produce significant differences, the average values were compared with those of 'Cannellini' and 'Borlotti' beans, the most known Italian landraces

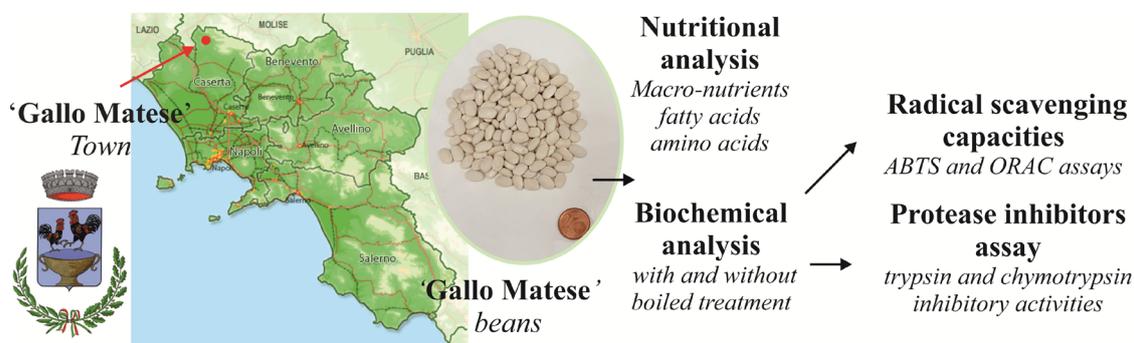


Fig. 1. Schematic workflow of the experimental strategy for the biochemical and nutritional characterization of ‘Gallo Matese’ beans

(Piergiorganni and Lioi, 2010), whose values are reported by the “Centro di Ricerca per gli Alimenti e la Nutrizione” (CREA, 2009).

The crude protein content of different types of beans represents between 16% and 33% of seeds (Azarpazhooh and Boye, 2012). In this study, the average amount of crude protein from “Gallo Matese” beans (22.64 g/100 g) was about 3% lower than ‘Cannellini’ beans (23.40 g/100 g) and about 12% higher than ‘Borlotti’ beans (20.20 g/100 g). Furthermore, the crude protein content of ‘Gallo Matese’ beans is similar to ‘Fagiolo occhio nero di Oliveto Citra’ beans (22.84 g/100 g) (Zaccardelli et al., 2013) and slightly

different with respect to several Campania bean landraces as ‘Dente di morto’, ‘Fagiolo di Controne’, ‘Mustacciello di Villaricca’, which have a crude mean protein content in a range between 20.70 and 25.85 g/100 g (Piergiorganni et al., 2015).

Lipid content (2.45 g/100 g) was higher than that reported for both ‘Cannellini’ (1.60 g/100 g) and ‘Borlotti’ (2.20 g/100 g), while in the literature data about Campania landraces are not available. In general, the amount retrieved is in accordance with the range (1.0–3.0%) of common beans (Azarpazhooh and Boye, 2012), confirming that dry beans are a low source of lipids.

Table 1. Nutritional values of ‘Gallo Matese’ and Italian common beans (CREA, 2009)

	‘Gallo Matese’ beans, g/100 g			Italian common beans, g/100 g	
	C ₁	C ₂	\bar{x}	Cannellini	Borlotti
Proteins	23.08 ±1.80	22.19 ±1.70	22.64	23.4	20.2
Lipids	2.49 ±0.54	2.40 ±0.34	2.50	1.6	2.2
Ash	4.31 ±0.03	4.24 ±0.04	4.80	n.r.	n.r.
Moisture	9.76 ±0.50	9.14 ±0.32	9.45	10.0	10.3
Starch	55.43 ±5.50	55.58 ±6.00	55.51	38.7	40.0
Glucose, mg/100 g	66.44 ±3.33	76.68 ±4.67	71.56	n.r.	n.r.
Carbohydrates	60.36	62.03	61.21	65.0	47.5

Values are means (±SD) of triplicate analyses ($n = 3$) and are expressed on a dry-weight basis. Data in the same row are not significantly different ($P > 0.05$) using the Bonferroni post-test. n.r. – not reported.

Carbohydrate content is 61.21% [range 55.0–65.0% (Azarpazhooh and Boye, 2012)], slightly lower than that reported for 'Cannellini' beans (about 6%), but higher with respect to 'Borlotti' beans (about 22%).

In addition, as reported in Table 1, the starch and free glucose content were determined. In particular, the starch content of 'Gallo Matese' beans (55.51 g/100 g) was higher than that reported for both 'Cannellini' (38.70 g/100 g) and 'Borlotti' (40.00 g/100 g). Considering the mean value of starch between 44.4% and 47.8% (Azarpazhooh and Boye, 2012) in common beans, 'Gallo Matese' beans have a higher content of this carbohydrate, although this may be influenced by the environmental conditions in which the plant grows and in which seed maturation occurs. Finally, the free glucose level of 'Gallo Matese' beans was 71.56 mg/100 g.

Amino acid content

Total amino acid content (free plus protein) from hydrolysate 'Gallo Matese' beans obtained by analysis of both C1 and C2 sites and their average (\bar{x}) is reported in Table 2, and no statistical differences were noted. Subsequently, the average values (\bar{x}) were compared with those of both 'Cannellini' and 'Borlotti' beans, showing qualitative and quantitative differences.

Glx (glutamic acid + glutamine; 3.42 g/100 g) was the most abundant among the total amino acids in 'Gallo Matese' beans, followed by arginine (1.76 g/100 g), Asx (aspartic acid + asparagine; 1.65 g/100 g), proline (1.51 g/100 g), phenylalanine (1.49 g/100 g) and serine (1.39 g/100 g), which represented about 53.1% of the total amino acids. In addition, the amount of essential amino acids [His, Ile, Leu, Lys, Met, Phe, Thr, Val; Trp (tryptophan is not included as it was not determined in the total hydrolysed samples – see Table 2)] in 'Gallo Matese' beans was 8.25 g/100 g (~39.0% of total). The amount of methionine and cysteine (as cysteine and cysteic acid) in 'Gallo Matese' beans is 0.86 g/100 g (~4.0% of total), confirming the low level of sulphur amino acids as reported for other legumes. On the other hand, the amount of sulphur amino acids in 'Cannellini' and 'Borlotti' beans is 0.45 g/100 g (2.08% of total) and 0.40 g/100 g (2.07% of total), respectively.

On the other hand, three Italian landraces revealed large amounts of the same amino acid, glutamic acid, which is 16.20%, 17.17% and 16.40% for 'Gallo

Matese', 'Cannellini' and 'Borlotti' beans, respectively. These data are similar to those reported by Neacsu et al. (2016), where common beans had a glutamic acid level of 16.31%. Furthermore, phenylalanine, threonine, arginine, proline, serine, and tyrosine in 'Gallo Matese' beans were higher than 'Cannellini', 'Borlotti' and data reported by Neacsu (2016). In terms of free amino acids, the total amount in 'Gallo Matese' beans was 388.39 mg/100 g on a dry-weight basis (Table 3). Glutamine was by far the most abundant among free protein amino acids (about 27.36% of total). Furthermore, aspartic acid (97.13 mg/100 g), asparagine (52.91 mg/100 g) and arginine (36.20 mg/100 g) were the most abundant free amino acids in 'Gallo Matese' beans, whereas the amount of each of the other protein amino acids did not exceed 95.86 mg/100 g of product (24.68% of free amino acids total content). The analysis also evidenced the presence of eleven non-protein amino acids [i.e. L- α -aminoadipic acid (AAAA); L- α -amminobutirrico (AABA); b-alanine (b-Ala); L-citrulline (Citr), L-cystathionine (Cysth); ethanolamine (Ethan); g-amino-n-butyric acid (GABA); phosphorylethanolamine (Pea); phosphoserine (Phser); L-sarcosine (Sarc); taurine (Taur)]. The amount of these non-protein amino acids was 20.09 mg/100 g (5.17% of total). The free amino acid content of 'Cannellini' and 'Borlotti' beans (retrieved in INRAN databank) and other landraces has not been published previously.

However, statistical analysis shows that significant differences between C1 and C2 samples are only present for asparagine or glutamine amino acids, which is most likely due to pedoclimatic differences between two sites and, in particular, nitrogen availability (Postles et al., 2016).

Fatty acid composition analysis

Common beans have a low total lipid content mainly comprising unsaturated fatty acids with a high percentage of both linoleic (C18:2, n-6) and linolenic (C18:3, n-3) fatty acids (Oomah et al., 2011).

The total saturated, monounsaturated and polyunsaturated fatty acid contents of 'Gallo Matese' beans, obtained by analyses of both C1 and C2 sites and their average (\bar{x}), are reported in Table 4. However, since the statistical analysis did not reveal significant differences, the average values were compared with those of three different varieties of Slovenian common beans,

Table 2. Total amino acid composition of ‘Gallo Matese’ and Italian common beans (CREA, 2009)

Amino acid	‘Gallo Matese’ beans, g/100 g – Italian common beans, g/100 g				
	C ₁	C ₂	\bar{x}	Cannellini	Borlotti
Essential amino acids					
His	0.77 ±0.07	0.67 ±0.04	0.72	0.66	0.60
Ile	0.89 ±0.08	0.75 ±0.02	0.82	1.24	1.07
Leu	1.47 ±0.14	1.13 ±0.05	1.30	0.72	1.63
Lys	1.45 ±0.16	1.14 ±0.05	1.30	1.64	1.44
Met	0.38 ±0.07	0.31 ±0.02	0.35	0.25	0.20
Phe	1.58 ±0.12	1.39 ±0.04	1.49	1.34	1.15
Thr	1.29 ±0.13	1.11 ±0.03	1.20	0.92	0.80
Trp	n.d.	n.d.	–	0.22	0.20
Val	1.15 ±0.11	0.98 ±0.03	1.07	1.38	1.07
Non-essential amino acids					
Ala	1.04 ±0.11	0.84 ±0.03	0.94	0.98	0.86
Arg	1.98 ±0.34	1.53 ±0.02	1.76	1.52	1.17
Asx	1.80 ±0.17	1.50 ±0.05	1.65	2.85	2.40
Cys	0.02 ±0.00	0.03 ±0.00	0.03	0.20	0.20
Cysteic acid	0.48 ±0.04	0.48 ±0.01	0.48	–	–
Glx	3.80 ±0.41	3.03 ±0.12	3.42	3.71	3.17
Gly	0.87 ±0.09	0.69 ±0.03	0.78	0.94	0.77
Pro	1.87 ±0.04	1.15 ±0.08	1.51	0.96	0.75
Ser	1.52 ±0.14	1.25 ±0.05	1.39	1.36	1.20
Tyr	0.95 ±0.09	0.86 ±0.03	0.91	0.72	0.65
Total	23.31	18.84	21.08	21.61	19.33

Values are means (±SD) of triplicate analyses ($n = 3$) and are expressed on a dry-weight basis.

Data in the same row are not significantly different ($P > 0.05$) using a Bonferroni post-test.

Protein amino acids. A three-letter code has been used: Asx – L-asparagine + L-aspartic acid, Arg – L-arginine, Cys – L-half cysteine, Glx – L-glutamine + 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, Ser – L-serine, Thr – L-threonine, Trp – L-tryptophan, Tyr – L-tyrosine, Val – L-valine.

n.d. – not determined.

Češnjevec, Semenarna_22 and Cipro beans (Pirman and Stibilj, 2003), given the absence of data in Italian “Centro di Ricerca per gli Alimenti e la Nutrizione” (CREA, 2009). The quantitative analysis of fatty acids

by using GC/MS revealed that ‘Gallo Matese’ beans are relatively low in lipids, with a saturated fatty acid content of only 19.7% (238 mg/100 g) and mainly consisting of unsaturated fatty acids. ‘Gallo Matese’

Table 3. Free amino acid composition of 'Gallo Matese' beans

Amino acid	'Gallo Matese' beans, mg/100 g		
	C ₁	C ₂	\bar{x}
Essential amino acids			
His	9.38 ±0.68	5.48 ±0.02	7.43
Ile	2.17 ±0.10	1.15 ±0.15	1.66
Leu	2.38 ±0.01	1.63 ±0.28	2.01
Lys	0.45 ±0.09	0.36 ±0.00	0.41
Met	17.37 ±1.20	13.93 ±0.23	15.65
Phe	6.31 ±0.38	2.89 ±0.02	4.60
Thr	2.64 ±0.31	1.30 ±0.02	1.97
Trp	5.88 ±0.47	2.23 ±0.03	4.06
Val	12.10 ±1.14	10.89 ±0.14	11.50
Non-essential amino acids			
AAAA	3.52 ±0.13	6.45 ±0.14	4.99
Aaba	0.33 ±0.03	0.07 ±0.00	0.20
β-ala	1.29 ±0.07	0.97 ±0.00	1.13
Ala	4.23 ±0.43	4.32 ±0.05	4.28
Arg	40.75 ±4.11	31.64 ±0.45	36.20
Asn*	73.35 ±5.13	32.47 ±0.67	52.91
Asp	91.69 ±7.32	102.57 ±8.2	97.13
Citr	0.42 ±0.05	0.25 ±0.06	0.34
Cys	0.76 ±0.04	0.54 ±0.09	0.65
Cysth	2.69 ±0.25	0.13 ±0.19	1.41
Ethan	2.74 ±0.09	2.31 ±0.18	2.53
GABA	0.26 ±0.04	0.42 ±0.05	0.34
Gln*	142.27 ±7.76	70.22 ±0.95	106.25
Glu	14.91 ±1.36	17.83 ±0.20	16.37
Gly	1.75 ±0.23	2.30 ±0.01	2.03
Pea	0.77 ±0.03	0.54 ±0.13	0.66
Phser	1.39 ±0.06	1.22 ±0.18	1.31
Pro	2.94 ±0.52	2.12 ±0.11	2.53
Sarc	5.65 ±0.26	2.99 ±0.01	4.32
Ser	2.08 ±0.31	1.35 ±0.08	1.72
Taur	0.91 ±0.41	1.42 ±0.07	1.17
Tyr	0.57 ±0.08	0.83 ±0.06	0.70
Total	453.95	322.42	388.39

Protein amino acids are reported in bold. Values are means (±SD) of triplicate analyses ($n = 3$) and are expressed on a dry-weight basis.

Data in the same row are not significantly different ($P > 0.05$) using the Bonferroni post-test.

*Very highly significant ($P < 0.001$).

beans contain relatively high levels of monounsaturated fatty acids (106 mg/100 g; 8.8% of total fatty acids content). The most abundant monounsaturated acid is oleic acid (C18:1, n-9), representing 8.1% of the total fatty acid content (98.03 mg/100 g), a higher percentage than those of Češnjevec, Semenarna_22 and Cipro beans (5.8%, 7.2% and 4.5%, respectively). Furthermore, the gondoic acid (C20:1, n-9) content of 'Gallo Matese' beans was 1.33 mg/100 g, whereas no traces of gondoic acid were detected in the three Slovenian bean varieties. Moreover, 'Gallo Matese' beans are an important source of essential polyunsaturated fatty acids, accounting for 71.5% of the total (864 mg/100 g). The principal polyunsaturated fatty acid in 'Gallo Matese' beans was α -linolenic acid (C18:3, n-3; 476.35 mg/100 g), which represents about 40% of the total fatty acid content, a value about 40% lower than those of the three Slovenian bean varieties. Polyunsaturated linoleic acid (C18:2, n-6; 388.23 mg/100 g) represents about 32% of total fatty acid content in 'Gallo Matese' beans, which is about 2.5% lower than Češnjevec beans and about 33% or 9% higher than Semenarna 22 or Cipro beans, respectively.

Finally, the balanced n-6 to n-3 ratio of 1:1 in 'Gallo Matese' beans ensures a good intake of polyunsaturated fatty acids, which are important for health and for the prevention and management of obesity (Simopoulos, 2016).

Anti-proteinase inhibitor activity

In general, beans contain several antinutritional compounds (ANCs), such as organic molecules or proteins (enzymes or protein inhibitors), whose assimilation has adverse effects on human health. Many of these ANCs are phytates, saponins, alkaloids, tannins, glycosides complex, lectins and proteinase inhibitors that interfere with the digestive processes (Krupa, 2008). In particular, protease inhibitors negatively affect the hydrolytic activities of proteases in protein intake, as a consequence of inhibition of proteolytic enzymes like trypsin and chymotrypsin (Sathe, 2012). Indeed, processes such as cooking or germination render these inhibitors inactivate, improving protein quality and increasing amino acid availability. In this framework, anti-trypsin and anti-chymotrypsin assays were carried out on a protein extract (106.8 mg/g of seeds) obtained from 'Gallo Matese' beans collected from the C1 site

Table 4. Fatty acid constituents of 'Gallo Matese' and Slovenian common beans (Pirman and Stibilj, 2003)

Fatty acid	'Gallo Matese' beans, mg/100 g			Slovenian common beans, mg/100 g			
	C ₁	C ₂	\bar{x}	Češnjevec	Semenarna 22	Cipro	
Saturated fatty acids							
Myristic	14:0	1.79 ±0.30	1.83 ±0.26	1.81	0.80	1.60	2.20
Pentadecylic	15:0	1.95 ±0.29	1.90 ±0.19	1.93	1.00	0.40	1.10
Palmitic	16:0	196.94 ±9.70	180.90 ±9.42	188.92	201.00	181.00	233.00
Margaric	17:0	2.46 ±0.47	2.65 ±0.48	2.54	2.70	3.10	3.10
Stearic	18:0	31.70 ±2.26	32.84 ±2.30	32.27	24.40	17.10	22.10
Arachidic	20:0	5.00 ±0.50	4.66 ±0.46	4.83	4.40	1.50	3.90
Behenic	22:0	5.46 ±0.59	5.65 ±0.62	5.56	3.50	10.60	<i>in trace</i>
			SFA, mg	238	238	215	266
Monounsaturated fatty acid							
Palmitoleic	16:1, n-7	5.89 ±0.63	5.46 ±0.52	5.68	3.60	3.10	3.20
Heptadecenoic	17:1, n-7	1.05 ±0.21	1.37 ±0.26	1.21	1.50	1.80	1.60
Oleic	18:1, n-9	99.02 ±7.08	97.04 ±6.69	98.03	86.60	102.00	66.50
Gondoic	20:1, n-9	1.40 ±0.37	1.25 ±0.29	1.33	n.d.	n.d.	n.d.
			MUFA, mg	106	92	107	71
Polyunsaturated fatty acid							
Linoleic	18:2, n-6	393.26 ±12.61	383.19 ±11.50	388.23	398.00	261.00	354.00
α-Linolenic	18:3, n-3	470.42 ±13.97	482.28 ±12.54	476.35	761.00	825.00	781.00
			PUFA	864	1159	1086	1135
			Total FA, g	1.21	1.49	1.41	1.47

Values are means (±SD) of triplicate analyses ($n = 3$) and are expressed on a dry-weight basis. Data in the same row are not significantly different ($P > 0.05$) using a Bonferroni post-test. n.d. – not determined.

and available in larger quantities for this biochemical characterization with and without boiling process. The inhibitory activity obtained from the protein extract (Fig. 2) was reported as IC₅₀ value. The anti-chymotrypsin activity of 'Gallo Matese' beans without thermal treatment (IC₅₀ 1.65 mg of raw protein extract) was ~26% higher than the anti-trypsin activity (IC₅₀ 2.21 mg of raw protein extract), while no protease activities were detected in the same experimental conditions. On the other hand, to confirm the decreasing effect of the

boiling process on trypsin inhibitor content, aliquots of 'Gallo Matese' seeds were boiled for 60 and 120 min, respectively, and their anti-protease activities were re-assayed. The protein extract from seeds after 60 and 120 min of boiling process showed a protein concentration of 3.20 and 2.00 mg/g of seeds, respectively. After a thermal treatment of 60 min, these results indicated that anti-chymotrypsin, as well as anti-trypsin activities were ~81% (IC₅₀ 8.70 mg of raw protein extract) and ~76% (IC₅₀ 9.40 mg of raw protein extract)

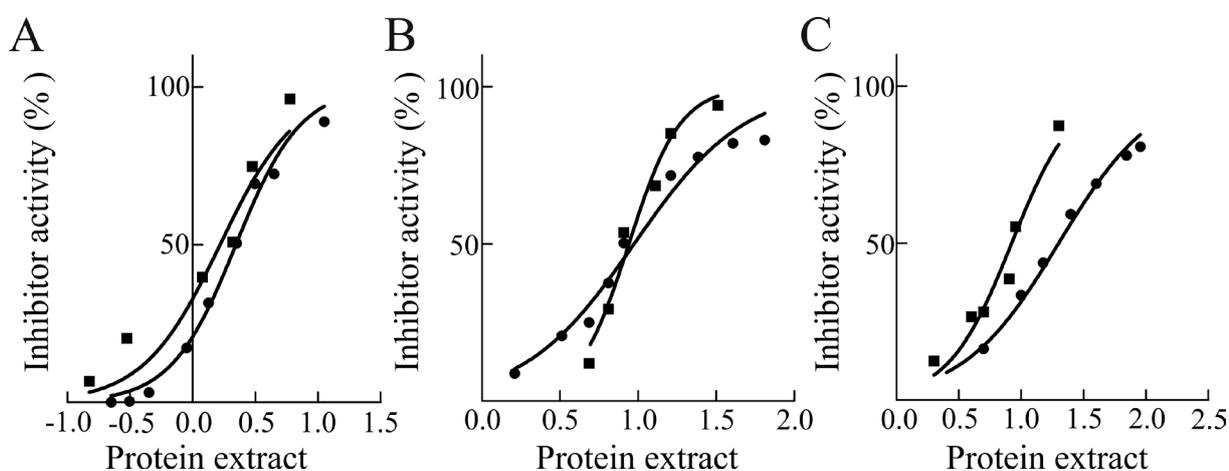


Fig. 2. Inhibition curves of trypsin (●) and α -chymotrypsin (■) by raw protein extract obtained from raw beans (A) or cooked beans at 1 hour (B) or 2 hour (C), respectively. Increasing concentrations of raw protein extract were added to a fixed concentration of enzymes as indicated in Material and methods

lower than those detected in raw bean seeds, respectively. On the other hand, anti-chymotrypsin and also anti-trypsin activities after 120 min were $\sim 80\%$ (IC_{50} 8.40 mg of raw protein extract) and $\sim 89\%$ (IC_{50} 20.00 mg of raw protein extract) lower than those detected in raw bean seeds, respectively. Therefore, as previously reported for other legumes, the boiling process reduces the anti-protease activities of 'Gallo Matese' beans. In addition, the thermal treatment has a higher effect on anti-trypsin with respect to anti-chymotrypsin activities. Therefore, this finding highlights the fact that boiled beans have a residual heat-resistant anti-chymotrypsin and anti-trypsin activity involved in the anti-cancer properties of legumes, due to carcinogenesis suppression, inhibiting proteolytic activities and/or expression of some proto-oncogenes as reported for colorectal cancer (Clemente and Arques, 2014; Lima et al., 2016).

Total phenol content and antioxidant capability of raw and boiled 'Gallo Matese' beans

Several epidemiological and clinical studies report the benefits of consuming legumes such as common beans due to the presence of phenolic compounds (Pauwels, 2011). Indeed, since polyphenols act as hydrogen donors, singlet oxygen quenchers or chelators, they are considered antioxidant agents (Zhang, 2015). Hence, we have determined the total polyphenol content

(TPC) from 'Gallo Matese' beans collected from C1 site available in larger quantities for this biochemical characterization, extracted by using a rotary shaker and acetone/water as extractants. The calculated TPC value was compared with that of beans previously boiled in water for 2 h, as occurs in the Mediterranean culinary tradition (see Methods). The polyphenol content of the boiling water was also measured. The TPC value of raw beans, 216.76 mg GAE per 100 g, was slightly higher than that of cooked beans (193.64 mg GAE per 100 g), whereas a poor amount of phenol substances was released in boiling water samples (15.76 mg GAE per 100 g; Fig. 3A). Thus, the effect of conventional boiling treatment seemed to have a weak influence on the 'Gallo Matese' beans' polyphenol content at atmospheric pressure. The variation of TPC is in agreement with previous studies, which found that boiling thermal treatment decreased the total phenolic content in vegetables due to the leaching of phenolic compounds into the cooking water, as well as the breakdown of these compounds during processing (Ismail et al., 2004; Xu and Chang, 2008). Interestingly, the lyophilized extract of cooked beans together with their cooking water displays a slight increase in the TPC, 276.16 mg GAE per 100 g (Fig. 3A).

The TPC of raw 'Gallo Matese' beans has also been compared in similar studies on different Italian *Phaseolus vulgaris* seeds by using the Folin-Ciocalteu

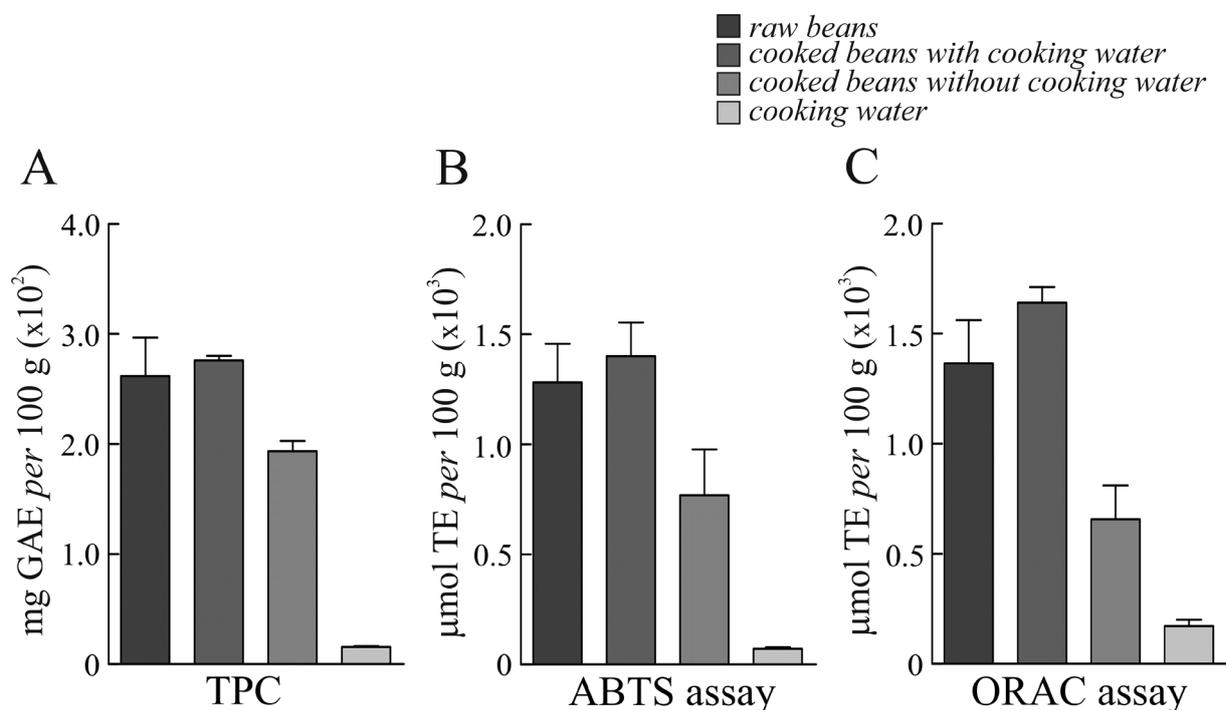


Fig. 3. Total phenol content and antioxidant capabilities in both raw beans, cooked beans with and without cooking waters and cooking water: A – total phenol content (TPC) method data, B – ABTS method data, C – ORAC method data. TPC are expressed as mg of gallic acid equivalents (GAE); ORAC and ABTS are expressed as μmol of trolox equivalents (TE) per 100 g on a dry-weight basis of each sample ±SD

method. It was observed that the TPC content of 'Gallo Matese' beans is lower with respect to 'Fagiolo occhio nero di Oliveto Citra' [310 mg GAE per 100 g (Zaccardelli et al., 2013)] seeds, but it is within the range 135–275 mg GAE per 100 g, as previously reported for other Italian white landraces (Ombra et al., 2016).

The evaluation of the antioxidant capability of the three extracts (raw seeds, cooked seeds with or without cooking water and cooking water) from 'Gallo Matese' beans was carried out by performing ABTS and ORAC methods. The data reveal that boiling treatment decreased 'Gallo Matese' beans' free radical scavenging efficacy (Fig. 3B, 3C). Interestingly, in this case the lyophilized extract of cooked beans together with their cooking water displays a slight increase in antioxidant capability. This increase is probably due to the cooking process, which could cause the thermal degradation of phytochemicals, but also the increase in antioxidant total content by enhancing their availability for extraction through the inactivation of polyphenol

oxidase, or the release of fiber-bound polyphenols into free polyphenols (Miglio et al., 2008).

CONCLUSIONS

Despite 'Gallo Matese' bean seeds having been consumed for centuries, there is still little information about their nutritional value. Today, interest in cultivation of this variety has increased because of health-conscious consumers becoming increasingly careful when choosing alternative healthy diets such as the Mediterranean diet.

Data obtained from our analysis emphasized that 'Gallo Matese' beans are a functional food, confirming that these seeds are rich in raw proteins and carbohydrates, and poor in lipids, mainly composed of unsaturated fatty acids. This finding provides additional information about both total and free amino acids, and total phenol content. Most importantly, the present work suggests radical scavenging activity, observed

even after the boiling process and partially in boiling water. This result confirms the healthy Italian habit of cooking *pasta e fagioli* in boiling water, preserving most of the radical scavenging activity. In contrast the process of boiling beans reduces proteinase inhibitors, such as anti-trypsin and anti-chymotrypsin, which interfere with digestive processes. On the other hand, beans that have undergone boiling treatment have residual heat-resistant anti-trypsin and anti-chymotrypsin activity, which is likely to be involved in the anti-cancer properties of legumes.

Overall, these results are useful for promoting the cultivation of 'Gallo Matese' beans and their consumption, due to their healthy food qualities. Furthermore, these data lay the basis for long-term agronomic experiments aimed at defining the goodness of this species compared to a standard species cultivated in the same area.

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