

Macro- and Micro-Nutrient Composition and Antioxidant Activity of Chickpea and Pea Accessions

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Key words: antioxidant activity, chickpeas, peas, phenolic extract, mineral composition, bioactive compounds

Epidemiological studies reported an inverse association between the consumption of legumes and the incidence of age-related diseases. This trend could be attributed to the presence of antioxidant compounds, especially phenolic and flavonoid compounds. In this paper, five pea (*Pisum sativum* L.) and twelve chickpea (*Cicer arietinum* L.) accessions, having different characteristics and geographical origin, were characterised in terms of antioxidant activity, as well as macro- and micro-nutrient composition. The antioxidant activity has been evaluated using both DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (2,2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) radical scavenging capacity assays. Chickpea and pea accessions showed a different behaviour in the presence of these different radicals. Chickpea accessions were characterised by significantly higher DPPH[•] scavenging activity, while peas showed a significantly higher value of antioxidant activity evaluated using the ABTS assay. Pea accessions had the highest content of total phenolic compounds, Zn, and Cu. A positive correlation was found between some minerals, such as Zn, Cu and P, and the ABTS^{•+} scavenging activity. Black and brown chickpea accessions showed significantly higher contents of anthocyanins, Mn, Mg, and Ca, which were positively correlated with the antioxidant activity assessed with the DPPH assay. Despite the dataset investigated in our study included a limited number of accessions, it was possible to highlight the influence of the chemical composition on the antioxidant activity due to the high phenotypic diversity found between the accessions, emphasising the importance of selecting the antioxidant activity assay according to the matrix to be evaluated.

INTRODUCTION

The demand for grain legume-based food is expected to increase in developing countries, as a consequence of the demographic growth, but also in the developed countries, given their contribution to a healthy diet and food safety. The increased awareness of risks associated with excessive consumption of animal proteins [Daryanto *et al.*, 2015] and with fat accumulation due to the high intake of energy-dense foods poor in micronutrients and bioactive compounds also plays a determinant role in prompting legume consumption.

In addition, greater cultivation and consumption of food legumes has high priority to increase the sustainability of agriculture in terms of soil fertility, greenhouse gas emissions, energy efficiency, pollution, and crop diversity [Annicchiarico, 2017].

Chickpea (*Cicer arietinum* L.) is the third grain legume species cultivated worldwide [FAOSTAT data, 2018]. It has been shown that the two commercial types of chickpea, *i.e.* *kabuli*, with large seeds and beige coat, and *desi*, with small seeds and dark-coloured, fall in different genetic clusters [De Giovanni *et al.*, 2017]. Furthermore, a black-pigmented chickpea type (*Apulian black*) traditionally cultivated in Apulia (Southern Italy), displayed peculiar phenotypic and genetic features [Pavan *et al.*, 2017]. From a nutritional point of view, chickpeas are characterised by high dietary fibre and lipid content [Jukanti *et al.*, 2012]. The lipid fraction, in particular for the coloured types such as *desi* and *Apulian black* type, has a high content of essential unsaturated fatty acids [Summo *et al.*, 2019a,b] which elicit beneficial effects on human health [Jukanti *et al.*, 2012].

Pea (*Pisum sativum* L.) is the fourth grain legume cultivated worldwide [FAOSTAT data, 2018], grown for both

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Submitted: 7 December 2020

Accepted: 15 April 2021

Published on-line: 19 May 2021

human nutrition and livestock feeding. Studies on pea genetic diversity highlighted clear differentiation between the pea wild progenitor, *P. sativum* subsp. *elatius*, and the main pea cultivated subspecies (*P. sativum* subsp. *sativum*). Within *P. sativum* subsp. *sativum*, geographical patterns of variation were identified, as landraces from the Mediterranean area, the Caucasus, Ethiopia, and Central Asia exhibited peculiar genetic features [Smykal *et al.*, 2012].

As other legumes, pea (*Pisum sativum* L.) and chickpea (*Cicer arietinum* L.) are characterised by low levels of lipids and high contents of proteins, complex carbohydrates, B group vitamins, and minerals. They represent a good source of minerals, such as iron, zinc, calcium, magnesium, potassium, sulphur, and selenium [Ashokkumar *et al.*, 2015] and carotenoids, such as β -carotene [Ashokkumar *et al.*, 2015].

Both pea and chickpea have been studied for agronomic [Fotiadis *et al.*, 2019], genetic [Pavan *et al.*, 2017], and nutritional features [Summo *et al.*, 2019a]. Furthermore, they have been proposed as functional ingredients of bakery products, such as bread and cakes [Millar *et al.*, 2019; Pasqualone *et al.*, 2019a], as well as ready-to-eat foods, such as purée and burgers [Summo *et al.*, 2016, 2019c].

Moreover, epidemiological studies reported an inverse association between the consumption of legumes and the incidence of age-related diseases [Kris-Etherton *et al.*, 2002]. The beneficial effect of legumes on health could be attributed to their content of phenolics and flavonoids [Fidrianny *et al.*, 2016], which are the most active antioxidant compounds in foods [Dudonne *et al.*, 2009]. Furthermore, antioxidant defences rely heavily on minerals in the diet, such as Fe, Mn, Cu, Zn, and Mg [Evans & Halliwell, 2001]. Dietary antioxidant compounds can stimulate cellular defences and help prevent oxidative damage [Dudonne *et al.*, 2009]. There are numerous published methods measuring the *in vitro* total antioxidant capacity. They can be classified in hydrogen atom transfer (HAT) or electron transfer (ET) based assays. The ET-based assays include the total phenols assay by Folin-Ciocalteu reagent, DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (2,2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) radical scavenging capacity assays [Huang *et al.*, 2005]. Both DPPH \cdot and ABTS $^{+\cdot}$ can be used to predict the antioxidant activity of vegetables, fruits, pulses, and food products [Fidrianny *et al.*, 2016; Yao *et al.*, 2013]. The principal aim of this study was to evaluate the antioxidant activity of chickpea and pea accessions by using two different radical scavenging capacity assays, such as DPPH and ABTS. Furthermore, proximate composition, contents of minerals, phenolic compounds, carotenoids, anthocyanins, and phytates of whole meal flour from the same accessions of chickpea and pea were determined. Finally, correlations between both antioxidant activities and legume flour composition were defined.

MATERIAL AND METHODS

Plant material and flour preparation

Twelve chickpea and five pea accessions were considered in this study, selected from the *ex situ* repositories of the United States Department of Agriculture (USDA),

TABLE 1. Chickpea (*Cicer arietinum* L.) and pea (*Pisum sativum* L.) accessions analysed.

Legume		Type	Seed size	Seed colour	Origin
Chickpea (<i>Cicer arietinum</i> L.)	PI292006	KC	Small	Beige	Jordan
	PI339154*	KC	Large	Beige	Turkey
	PI357648*	KC	Small	Beige	Serbia-Montenegro
	PI518255*	DC	Small	Brown	Afghanistan
	PI251514*	DC	Small	Black	Iran
	PI140293*	DC	Small	Brown	Iran
	PI358934*	DC	Small	Black	Iran
	PI533683*	DC	Large	Black	Spain
	W610046*	DC	Large	Black	Bulgaria
	110694*	AB	Large	Black	Italy
	MG_13*	AB	Large	Black	Italy
	MG_17*	AB	Large	Black	Italy
	Pea (<i>Pisum sativum</i> L.)	IG116297		Medium	Green
ROR12			Large	Green	Italy
IG52442			Medium	Green-pigmented	Syria
IG134828			Medium	Green-pigmented	Georgia
IG51520			Medium	Green-pigmented	Ethiopia

KC – *kabuli* chickpea, DC – *desi* chickpea, and AB – *Apulian black* chickpea.

*The chickpea accessions are part of a wide collection already characterised, whose data repository is in Summo *et al.* [2019a].

the Department of Plant, Soil and Food Science of the University of Bari, Italy (DiSSPA), and the Institute of Biosciences and Bioresources of the Italian National Research Council (CNR-IBBR) (Table 1). For each, type, country of origin, and phenotypic traits (seed size and colour) were indicated. Chickpea accessions encompassed the three genetic clusters previously identified, corresponding to the *desi*, *kabuli*, and *Apulian black* types [Pavan *et al.*, 2017]. Genetic diversity of pea germplasm was ensured by selecting one accession referable to the wild progenitor *P. sativum* subsp. *elatius* collected in Syria, and four *P. sativum* subsp. *sativum* accessions originating from the Mediterranean area (Italy and Turkey), the Caucasus (Georgia), and Ethiopia. Among them, the Italian landrace ROR12 was reportedly resistant to the parasitic weed *Orobancha crenata* [Pavan *et al.*, 2016]. All plants were grown in the experimental farm “P. Martucci” of the University of Bari “Aldo Moro”, Italy (41°01'22.1" N, 16°54'21.0" E) during the growing season 2017–2018. They were harvested according to a randomised complete block design with two replicates, each replicate being formed by 30 individual plants. After harvesting at crop maturity, chickpea and pea seeds were milled (ETA mill, Vercella Giuseppe, Mercenasco, Italy) and sieved at 0.6 mm.

Preparation of extracts and determination of the antioxidant activity

Antioxidant activity was determined using both DPPH and ABTS radical (DPPH[•] and ABTS^{•+}) scavenging capacity assays. The determinations were performed for an aqueous-methanol extract (20/80, v/v) prepared as reported by Summo *et al.* [2019b]. The DPPH radical scavenging capacity assay was carried out following the procedure described in Pasqualone *et al.* [2015]. The ABTS assay was performed according to Difonzo *et al.* [2017]. The antioxidant activity values were expressed as μmol of Trolox equivalent per g of dry matter (d.m.) of seeds. Each analysis was done in triplicate.

Determination of nutritional composition and bioactive compounds in flours

Proteins (total nitrogen \times 5.7), lipids, ashes, total dietary fibre, and moisture of the flours were determined according to the Association of Official Analytical Chemists (AOAC) methods 979.09, 945.38 F, 923.03, 991.43 and 925.10, respectively [AOAC, 2006]. Lipid content was determined. Carbohydrate content was calculated by difference.

Total carotenoid content was assessed using the method reported by Pasqualone *et al.* [2013] and was expressed as mg of β -carotene equivalent per kg of seed d.m.

Total anthocyanin content was determined as described by Pasqualone *et al.* [2015] and was expressed as mg of cyanidin 3-*O*-glucoside equivalent per kg of seed d.m.

Total phenolic compound (TPC) content was assessed as described by Summo *et al.* [2019b] using the extracts prepared as previously reported in section *Preparation of extracts and determination of the antioxidant activity*. The content of total phenolic compounds was expressed as mg of ferulic acid equivalent per g of seed d.m., considering a calibration curve prepared with ferulic acid at different concentrations.

Total phytate content was measured according to the method reported in Summo *et al.* [2019b].

Determination of minerals in flours

The determination of minerals in flours was performed by digesting using a microwave oven (CEM 6, Mars, CEM Corporation, Matthews, United States). Briefly, 0.5 g of each sample was weighed into a Teflon vessel, and 7 mL of HNO₃ (65%) and 1 mL of H₂O₂ (30%) were added [Rybicka & Gliszczynska-Świgło, 2017]. After cooling, digests were diluted to 50 mL with demineralised water (Hydrolab System, Wiślna, Poland) and kept at 4°C until spectroscopic determinations. Three digests were prepared for each sample. Spectroscopic determinations of minerals were performed using atomic emission spectroscopy and the method described in detail by Ozbek & Akman [2016]. Analytical wavelengths for minerals were: 213.9 nm for Zn, 324.8 nm for Cu, 372.0 nm for Fe, 403.1 nm for Mn, 616.2 nm for Ca, 404.4 nm for K, and 589.0 nm for Na. The spectroscopic analysis was performed using two independent standard curves with a range from 0.05 to 1 $\mu\text{g}/\text{mL}$ for microelements, 0.05 to 5 $\mu\text{g}/\text{mL}$ for Na, and from 10 to 100 $\mu\text{g}/\text{mL}$ for other macroelements. Due to the high limit of quantification (LOQ) of phosphorus in atomic spectroscopy, its content was determined using the spectrophotometric molybdenum blue method adopted for multiple analysis

using 48-microwell plates and microplate spectrophotometer (BioTek PowerWave XS2, Biokom, Warsaw, Poland) [Murphy & Riley, 1962]. Briefly, 0.16 mL of the sample, then 0.08 μL of 5% ammonium molybdate, 0.08 μL of 0.5% hydroquinone, and 0.08 μL of 20% sodium sulphite were added to the well. The plate was shaken and left for 30 min in the dark; the absorbance was measured at 823 nm.

Statistical analysis

Data were subjected to one-way ANOVA followed by Tukey's HSD test, considering both the differences between the species (chickpea vs. pea) and those among the accessions. Significant differences among the values of all recorded variables were determined at $p < 0.05$ by the XLStat software (Addinsoft SARL, New York, NY, USA). Correlation analysis was performed by the same software.

RESULTS AND DISCUSSION

Antioxidant activity evaluation

The antioxidant activity has been evaluated using two different radical scavenging capacity assays, namely DPPH and ABTS, and expressed as μmol Trolox/g of dry matter (Figure 1). A different activity was observed between chickpea and pea accessions depending on the assay. Chickpea accessions were characterised by a significantly higher DPPH[•] scavenging activity, while peas showed a significantly higher value of antioxidant activity when the ABTS assay was performed. The same trend has been reported by other researchers in green bean (*Phaseolus radiates* L.) and peanut (*Arachis hypogaea* L.) extracts [Fidrianny *et al.*, 2016]. This result could be linked to the different chemical composition that characterised the two different legume species analysed. In fact, it has been shown that different phenolic compounds are responsible for quenching different free radicals [Xu *et al.*, 2016]. In order to explain the different antioxidant activities between chickpea and pea species, correlations between *in vitro* radical scavenging capacity and compositional features of legume accessions were investigated.

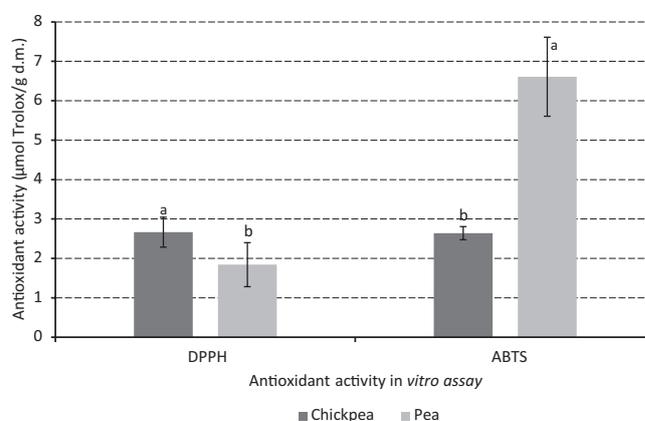


FIGURE 1. Mean values, standard deviation and results of the statistical analysis of antioxidant activity of chickpea and pea accessions assessed using both DPPH and ABTS radical scavenging assays. Different small letters indicate significant differences between the species ($p < 0.05$) for the type of antioxidant activity method used ($n = 12$ for chickpeas and $n = 5$ for peas).

TABLE 2. Proximate composition and content of bioactive compounds in the chickpea (*Cicer arietinum* L.) and pea (*Pisum sativum* L.) accessions analysed.

	Variety	Type	Proteins (g/100 g d.m.)	Lipids (g/100 g d.m.)	Ashes (g/100 g d.m.)	Carbohydrates (g/100 g d.m.)	Total dietary fiber (g/100 g d.m.)	Total phenolic compounds (mg ferulic acid/g d.m.)	Total carotenoids (mg β -carotene/kg d.m.)	Total anthocyanins (mg cyanidin 3-O-glucoside/kg d.m.)	Total phytates (mg phytic acid/g d.m.)
Chickpea*	MG_13	AB	20.29	3.36	3.37	54.32	18.67	1.08	34.21	121.99	14.97
	MG_17	AB	17.31	4.42	2.69	59.86	15.72	0.82	41.26	79.16	9.66
	110694	AB	22.71	3.35	3.90	52.84	17.20	0.96	42.76	119.00	11.36
	PI339154	KC	18.11	2.86	3.45	66.36	9.21	0.92	27.76	25.78	11.86
	PI292006	KC	20.34	4.16	3.64	60.60	11.26	0.69	29.46	32.45	14.72
	PI357648	KC	21.38	4.47	3.48	62.73	7.94	0.81	22.76	27.45	10.35
	PI518255	DC	17.80	2.83	3.75	45.81	29.82	0.71	46.85	44.37	15.29
	PI251514	DC	19.17	3.71	3.70	51.08	22.35	0.79	41.61	159.62	13.96
	PI140293	DC	19.32	2.80	3.47	49.39	25.03	1.08	26.01	46.52	11.79
	PI358934	DC	17.30	3.90	3.48	55.36	19.95	0.76	37.03	155.79	15.44
	PI533683	DC	20.19	3.35	3.70	52.70	20.06	1.04	42.27	115.40	11.02
	W610046	DC	25.92	3.41	3.65	48.87	18.15	0.83	48.92	103.23	11.95
Mean			19.98 ^B	3.55 ^A	3.52 ^A	54.99 ^A	17.95 ^A	0.87 ^B	36.74 ^A	85.90 ^A	12.70 ^A
DS			2.50	0.59	0.30	6.20	6.37	0.14	8.59	49.63	2.06
Pea	IG116297		26.82	1.68	2.89	50.95	17.66	1.12	25.03	33.36	13.23
	ROR12		24.42	1.52	3.32	58.07	12.67	1.22	16.72	19.26	10.71
	IG52442		27.76	1.63	3.68	47.84	19.09	1.10	59.39	78.30	14.78
	IG134828		26.75	2.06	3.45	53.69	14.04	1.05	33.59	72.63	13.99
	IG51520		26.02	1.30	3.49	55.56	13.63	1.03	23.56	35.92	16.09
Mean			26.36 ^A	1.64 ^B	3.36 ^A	53.22 ^A	15.42 ^A	1.10 ^A	31.66 ^A	47.89 ^A	13.76 ^A
DS			1.25	0.28	0.30	3.98	2.79	0.07	16.62	26.03	2.01

KC – *kabuli* chickpea, DC – *desi* chickpea, and AB – *Apulian black* chickpea.

Different letters indicate significant differences between the species at $p < 0.05$. *The chickpea accessions are part of a wide collection already characterised, whose data repository is in Summo *et al.* [2019a].

Nutritional composition and bioactive compound content

Table 2 reports proximate composition and content of bioactive compounds of chickpea and pea accessions examined in this study. Significant differences ($p < 0.05$) between the two species were found for protein, lipid, and carbohydrate contents. Chickpea showed a significantly higher lipid content and significantly lower protein and carbohydrate contents than pea. No significant differences ($p > 0.05$) between species emerged for total dietary fibre content, possibly due to the high variability observed among the accessions within the species.

Data on the chemical composition and bioactive compound content of a collection of chickpea accessions, including the twelve ones tested in this study, were reported and discussed in our previous work [Summo *et al.*, 2019b]. Considering the data on the pea accessions (Table 2), a large variation was currently observed among the pea accessions, especially for bioactive compounds. In particular, the accession ROR12 showed the highest value of total

phenolic compounds (1.22 mg ferulic acid/g d.m.). The observed mean value of total phenolic compounds was higher than the levels obtained by Zia-Ul-Haq *et al.* [2013], who reported 0.99 mg/g as a maximum value of total phenolic compounds detected in the cultivar Climax. Notably, at a high concentration, antioxidants can act as pro-oxidants by reacting with molecular oxygen [Sotler *et al.*, 2019]. Due to the high metabolic rate, reactive oxygen species (ROS) generation is incredibly high in transformed cells [Perillo *et al.*, 2020].

The total carotenoid content also varied considerably among pea accessions, ranging from 16.72 mg β -carotene/kg d.m. in the accession ROR12 to 59.39 mg β -carotene/kg d.m. in the IG52442. Ashokkumar *et al.* [2015], examining a collection of 94 pea genotypes, found a carotenoid content in pea ranging from 10 to 27 μ g/g in accessions with green cotyledons, and from 5 to 17 μ g/g in accessions with yellow cotyledons.

TABLE 3. Mineral composition of the chickpea (*Cicer arietinum* L.) and pea (*Pisum sativum* L.) accessions analysed.

	Variety	Type	Zn (mg/100 g d.m.)	Cu (mg/100 g d.m.)	Fe (mg/100 g d.m.)	Mn (mg/100 g d.m.)	Mg (mg/100 g d.m.)	Ca (mg/100 g d.m.)	K (mg/100 g d.m.)	Na (mg/100 g d.m.)	P (mg/100 g d.m.)
Chickpea	MG_13	AB	4.46	1.03	4.22	2.48	176.49	194.69	898.45	4.89	405.82
	MG_17	AB	2.81	0.29	3.78	4.78	153.95	290.81	756.18	2.89	297.10
	110694	AB	2.65	0.37	4.26	3.63	184.57	219.68	1055.07	7.00	425.91
	PI339154	KC	2.80	0.43	2.95	3.40	173.57	146.50	1159.11	7.53	456.10
	PI292006	KC	2.80	0.31	3.52	3.07	168.78	211.94	1009.69	9.09	406.16
	PI357648	KC	2.49	0.23	2.73	3.26	160.20	174.71	957.08	9.99	386.94
	PI518255	DC	3.05	0.36	4.22	4.32	179.79	431.83	1034.08	15.48	419.95
	PI251514	DC	2.15	0.25	4.11	3.66	183.79	334.91	1027.31	39.36	394.75
	PI140293	DC	2.38	0.20	3.20	3.84	188.73	279.21	984.70	19.91	386.19
	PI358934	DC	2.25	0.22	3.44	4.34	190.05	376.76	1005.02	16.69	364.63
	PI533683	DC	2.35	0.23	3.68	4.03	179.16	305.03	980.09	1.83	395.08
	W610046	DC	1.99	0.21	3.25	3.32	175.69	210.17	1007.91	2.11	395.15
<i>Mean</i>			2.68 ^B	0.34 ^B	3.61 ^B	3.68 ^A	176.22 ^A	264.69 ^A	989.56 ^A	11.40 ^A	394.48 ^A
<i>DS</i>			0.64	0.23	0.52	0.63	10.92	86.70	95.99	10.59	38.37
Pea	IG116297		3.74	0.87	4.00	0.96	157.63	117.07	884.55	3.14	474.17
	ROR12		3.16	0.56	3.69	1.12	153.90	90.96	937.62	5.02	412.93
	IG52442		4.39	0.84	5.23	1.07	177.15	119.61	996.19	2.97	543.83
	IG134828		4.08	0.72	4.66	0.99	163.56	97.07	979.05	3.15	503.97
	IG51520		2.56	0.70	3.87	1.02	166.99	129.17	971.88	1.64	421.20
<i>Mean</i>			3.58 ^A	0.74 ^A	4.29 ^A	1.03 ^B	163.85 ^B	110.78 ^B	953.86 ^A	3.18 ^A	471.22 ^A
<i>DS</i>			0.73	0.12	0.64	0.06	9.01	16.10	44.21	1.21	55.35

KC – *kabuli* chickpea, DC – *desi* chickpea, and AB – *Apulian black* chickpea. Different letters indicate significant differences between the species at $p < 0.05$.

The total anthocyanin content varied from 19.26 mg cyanidin 3-*O*-glucoside/kg d.m. in the non-pigmented pea accession ROR12 to 78.30 mg cyanidin 3-*O*-glucoside/kg d.m. in the pigmented accession pea IG52442. Notably, anthocyanin content was highly variable even within pigmented accessions with the minimum value (23.56 mg cyanidin 3-*O*-glucoside/kg d.m.) displayed by the accession IG 51520.

Legumes contain non-nutritional factors, such as phytates, that can reduce the bioavailability of some compounds or inhibit the enzymes necessary for their digestion [Shi *et al.*, 2018]. As reported in Table 2, no significant differences were observed between the two species, although considerable variation was found among the individual accessions. Pea accessions under the study showed a higher content of phytic acid than green and yellow peas studied by other researchers. Millar *et al.* [2019] reported that phytic acid content was 543.41 mg/100 g in the green pea and 574.14 mg/100 g in the yellow one was 574.14 mg/100g [Millar *et al.*, 2019].

Phytic acid is the principal storage form of phosphorus in seeds; this compound and its salts are capable of forming complexes with minerals, such as Ca, Cu, Mg, Fe, and Zn, thereby having a negative effect on their gastrointestinal absorption [Shi *et al.*, 2018].

Mineral composition

Potassium (K) was the most abundant mineral found in both chickpeas and peas (Table 3), without significant differences between them. Instead, significant differences were observed for other minerals such as zinc (Zn), copper (Cu), iron (Fe), manganese (Mn), magnesium (Mg), calcium (Ca), and phosphorus (P) ($p < 0.05$). Chickpeas had the highest contents of Mn, Mg, and Ca. Magnesium and calcium contents in chickpeas were higher than those (mean values of 1402 and 1040 $\mu\text{g/g}$, respectively) reported in Vandemark *et al.* [2018], but similar to those reported by Kaya *et al.* [2018]. Peas had significantly higher Zn, Cu, Fe, and P values

TABLE 4. Correlation table (correlation coefficient – r values) between the *in vitro* antioxidant activity (DPPH and ABTS assays) and chemical compound contents determined in the pulse accessions.

	Pr	L	A	C	DF	TPC	TC	TA	PH	Zn	Cu	Fe	Mn	Mg	Ca	K	Na	P
DPPH	-0.53	0.58	-0.04	-0.14	0.34	-0.31	0.66	0.64	0.13	-0.11	-0.13	0.14	0.64	0.45	0.53	-0.20	0.04	-0.48
ABTS	0.73	-0.79	-0.46	-0.02	-0.26	0.59	-0.50	-0.43	0.01	0.59	0.61	0.39	-0.86	-0.59	-0.68	-0.26	-0.31	0.70

Pr – Proteins; L – Lipids; A – Ashes; C – Carbohydrates; DF – Dietary fibers; TPC – Total phenolic compounds; TC – Total carotenoids; TA – Total anthocyanins; PH – Phytates; r values in bold indicate a significant correlation ($p < 0.05$).

than chickpeas. The values found in our study agree with Wang & Daun [2004], who reported a range of 2.50–5.20, 0.40–0.90, 4.30–7.90, and 270.30–950.50 mg/100 g for Zn, Cu, Fe, and P, respectively.

At the intraspecific level, *Apulian black* and some *desi* chickpea accessions showed a high Fe content, with the highest value observed in the accession 110694 (4.26 mg/100 g d.m.). In contrast, *kabuli* chickpeas were characterised by a low Fe content, with the lowest value found in the accession PI357648 (2.73 mg/100 g d.m.). In accordance with the previous study of Jukanti *et al.* [2012], DC accessions showed the highest Ca content (mean value 322.99 mg/100 g d.m.), followed by AC (mean value 235.36 mg/100 g d.m.) and *kabuli* (mean value 177.72 mg/100 g d.m.). The DC accession PI518255 displayed the highest Ca content (431.83 mg/100 g d.m.), which was found to be far from the mean value shown for chickpeas (264.69 mg/100 g d.m.). The AB variety MG_13 had the highest Zn and Cu levels (4.26 and 1.03 mg/100 g d.m., respectively).

Peas were characterised by a low variability among different accessions. However, the pigmented variety IG51520 had the highest content of Ca (129.17 mg/100 g d.m.), which was higher than the maximum value (106.90 mg/100 g) reported by Wang & Daun [2004].

Correlations between antioxidant activity and flour composition

Correlations between *in vitro* radical scavenging capacity and compositional features of legume accessions are reported in the Table 4.

A positive correlation was observed between the total phenolic content and ABTS^{•+} scavenging activity ($r=0.59$; $p < 0.05$). On the contrary, a negative, but not significant ($p > 0.05$) correlation was observed between ABTS assay results and contents of both carotenoids and anthocyanins. Considering the same compounds, an inverse trend was found for DPPH[•] scavenging activity. Bioactive compounds, such as phenolics, carotenoids, and anthocyanins, are recognised as antioxidants. Thus, they can prevent or reduce lipid peroxidation and scavenge free oxygen radicals through their high antioxidant activities [Ashokkumar *et al.*, 2015]. Moreover, phenolic compounds exhibit anti-tumoral, anti-inflammatory, and anti-allergic properties while anthocyanins are important due to their anti-carcinogenic properties and the ability to limit the incidence of hepatic steatosis and cardiovascular diseases, to control obesity, and to mitigate diabetes [Hernandez-Velazquez *et al.*, 2020]. Interestingly a significant association has been found between the total flavonoid intake

and a high level of magnesium, paralleled by a reduction of the metabolic syndrome [Jin *et al.*, 2020].

By contrast, several flavonoids, known for their antioxidant features, were proved, instead, to act as prooxidants and mutagenic factors in the *in vitro* studies [Rahal *et al.*, 2014]. A study conducted to determine the potential of grape pomace extracts as a source of natural antioxidants reported a positive correlation between ABTS^{•+} scavenging capacity and total phenolic contents, as well as with the total flavonoid contents [Xu *et al.*, 2016]. Yao *et al.* [2013] confirmed these results in black mung beans, where a significant positive correlation was found between bound phenolic acids and ABTS^{•+} scavenging activity ($r=0.941$; $p < 0.01$). Flavonoids and tannins have a relevant influence on the ABTS antioxidant activity, while anthocyanin compounds give a greater contribution to the antioxidant capacity measured by DPPH test, as shown by Xu *et al.* [2016] in pomace extracts. Furthermore, a negative correlation between ABTS^{•+} scavenging activity and β -carotene was reported by Thaipong *et al.* [2006] for methanol extracts from guava fruit. Considering the nutritional composition, the ABTS data showed a positive correlation with protein content ($r=0.73$; $p < 0.01$), whereas the same data were negatively correlated with lipid content ($r=-0.79$; $p < 0.01$) (Table 4). As previously reported by other researchers in leguminous seeds [Grela *et al.*, 2017], a positive correlation was found between DPPH[•] scavenging activity and lipid contents ($r=0.58$; $p < 0.05$). Grela *et al.* [2017] have reported a high correlation between DPPH[•] scavenging activity and unsaturated fatty acid contents, especially polyunsaturated ones, in several legumes, namely lupines, peas, chickpeas, lentils, grass peas, and common beans. In contrast, no correlation between fatty acids and DPPH[•] scavenging activity in 20 Canadian lentils cultivars was reported by Zhang *et al.* [2014]. Usually, the number of unsaturated bonds in the fatty acids induces an exponential increase in the susceptibility to oxidation. Therefore, the content of the individual double bonds of fatty acids may not be directly related in a linear way to the antioxidant activity. The positive correlation between the antioxidant activity (measured by DPPH test) and lipid content found in our study may suggest that other compounds have a significant influence on the resistance to oxidation of fatty acids [Grela *et al.*, 2017]. For instance, as mentioned above, carotenoids, anthocyanins, and phenolic compounds can contribute to the increase in the antioxidant potential.

A significant negative correlation was found between ABTS^{•+} scavenging activity and Mn ($r=-0.86$; $p < 0.01$), Mg ($r=-0.59$; $p < 0.05$) and Ca ($r=-0.68$; $p < 0.01$), whereas

the correlation was positive for Zn ($r=0.59$; $p<0.05$), Cu ($r=0.61$; $p<0.05$) and P ($r=0.70$; $p<0.01$). No significant correlation between DPPH[•] scavenging activity and mineral compounds was found, except for Mn ($r=0.64$; $p<0.05$). Despite the large amount of information available in scientific literature on mineral content of legumes, to the best of our knowledge, there are no reports on the direct correlation between mineral content and antioxidant activity. However, several studies suggested that an imbalance of minerals would change the content of polyphenols and flavonoids [Grela *et al.*, 2017; Sulaiman *et al.*, 2011]. This behaviour may explain the positive correlation found for the content of Mn and DPPH[•] scavenging activity. In fact, Mn is involved in activating enzymes that enhance the biosynthesis of flavonoids [Gordon, 2007]. A significant correlation between Mn content and DPPH[•] scavenging activity was reported by Sulaiman *et al.* [2011] in banana (*Musa* sp.) fresh pulps and peels. Furthermore, Zn-deficient or Zn-excess conditions cause changes in the antioxidant enzyme activities, as shown in bean plants by Prabhu Inbaraj & Muthuchelian [2011]. Tewari *et al.* [2006] reported, instead, an increase in the activity of the antioxidative enzyme superoxide dismutase (SOD) in mulberry (*Morus rubra* L.) Mg-deficient plants, suggesting an inverse relationship between Mg and antioxidant activity. Other researchers reported a significant correlation between the total flavonoid content and minerals due to the chelating role of polyphenols, especially condensed tannins [Rehecho *et al.*, 2011]. Therefore, these compounds may prevent or delay metal-catalysed initiation and decomposition of lipid hydroperoxides. Rehecho *et al.* [2011] reported significant correlations between the total flavonoid content and minerals, such as K, Zn, Cu, Ca, and Mg in verbena extracts.

CONCLUSION

Chickpea and pea accessions showed a different anti-radical activity against DPPH[•] and ABTS^{•+}. In particular, chickpea accessions were characterised by significantly higher DPPH[•] scavenging activity, while pea showed a significantly higher value of antioxidant activity evaluated using the ABTS assay.

Pea accessions had the highest content of total phenolic compounds, Zn and Cu. A positive correlation was found between some minerals, such as Zn, Cu and P, and the ABTS^{•+} scavenging activity found. Black and brown chickpea accessions showed a significantly higher content of anthocyanins, Mn, Mg and Ca, which were positively correlated with the antioxidant activity assessed by the DPPH assay. Therefore, the high phenolic content found in pea accessions was linked to the higher ABTS^{•+} scavenging capacity, while chickpeas, especially *Apulian black* and *desi* types, having high carotenoid and anthocyanin contents, were able to quench the DPPH radical.

Furthermore, the content of minerals and their composition may influence the antioxidant activity, especially ABTS^{•+} scavenging. Indeed, a significant negative correlation was found between ABTS^{•+} scavenging activity and Mn, Mg, and Ca, whereas the correlation was positive for Zn, Cu, and P.

Despite the dataset investigated in our study included a limited number of accessions, it was possible to highlight the influence of the chemical composition on the antioxidant activity due to the high phenotypic diversity found between the accessions, emphasising the importance of selecting the antioxidant activity assay according to the matrix to be evaluated.

RESEARCH FUNDING

This research has been performed within the project “LEGume GENetic RESources as a tool for the development of innovative and sustainable food TEchnological system” supported under the “Thought for Food” Initiative by Agropolis Fondation (through the “Investissements d’avenir” programme with reference number ANR-10-LABX-0001-01), Fondazione Cariplo, and Daniel & Nina Carasso Foundation.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

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