Journal of Food Biochemistry ISSN 1745-4514

ANTIOXIDATIVE ACTIVITY IN THE SEEDS OF 28 *VICIA* SPECIES FROM SOUTHERN SPAIN

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Accepted for Publication December 16, 2008

doi:10.1111/j.1745-4514.2010.00459.x

ABSTRACT

The antioxidant activity of the polyphenols extracted from the seeds of 28 *Vicia* species collected in southern Spain was investigated. The seeds were all taken from wild populations, although some of these species are or have been used to feed livestock and for crop rotation. The polyphenol concentration in ground seeds, ranging from 1.9 to 21.3 mg/g, was generally lower in populations belonging to species that were cultivated. The highest specific polyphenols antioxidant activity (referred to as catechin equivalents in the extracts) was observed in the extracts from *Vicia parviflora* and *Vicia tenuifolia*. On the contrary, the highest total antioxidant activity (referred as to extract volume) was observed in *Vicia sativa*. The antioxidant activity in these extracts was at least twice the observed in chickpea, lupin and soy extracts. These results suggest that these easy to grow *Vicia* species may represent a source of natural polyphenols with high antioxidant activity.

PRACTICAL APPLICATIONS

The polyphenols content and antioxidative activity in 28 species of *Vicia* (*Fabaceae*) have been studied. *Vicia* genus is a group of legumes with a high potential interest in human nutrition. In this work, we show that, in addition to their recognized good nutritional properties, *Vicia* contains polyphenols with a high antioxidant activity, even higher than the observed in commercial legumes such as chickpea, lupin or soy. Hence, it is shown that *Vicia* seeds are a source of functional compounds, such as antioxidant polyphenols, that may have health-promoting properties resulting in a revalorization of these crops.

INTRODUCTION

The common feature of all polyphenols, including phenolic acids, flavonoids, lignans and stilbenes, is a hydroxy-substituted benzene ring (Manach *et al.* 2004). Legumes are especially rich in flavonoids, which include flavanols, flavones, flavanones, flavonols, isoflavonoids and anthocyanins (Ramos 2007). A variety of biological functions have been proposed for polyphenols in plants, such as protection against radiation, oxidative damage and diseases, as well as participation in signaling pathways (Parr and Bolwell 2000). The antioxidant properties of polyphenols have been associated with the health-promoting effects of diets rich in fruits and vegetables (Balasundram *et al.* 2006). These health-promoting properties include reduced risk from cancer, cardiovascular and neurodegenerative diseases (Ramassamy 2006; Boudet 2007; Seifried *et al.* 2007). In addition, many polyphenols have been shown to have antiproliferative activity and to induce apoptosis (Pan *et al.* 2008).

Grain legumes are crops of great economic and nutritional importance, and the demand for them is expected to increase in coming years (Duranti 2006). Some of these grain legumes are staples in the traditional diets of many parts of the world, particularly in developing countries, including soy and *Phaseolus* spp. in Asian and Latin-American countries, respectively. Legumes contain a variety of phytochemicals that may have health-promoting effects if consumed on a regular basis, including a reduced risk of developing

diabetes mellitus, coronary heart disease and colon cancer (Tharanathan and Mahadevamma 2003).

The economical and nutritional value of grain legumes can be improved through extraction of their bioactive components which may be useful in the formulation of foods with improved nutritional, functional or health-promoting properties. This is especially true for grain legumes that are undervalued. A great deal of phytodiversity has been lost in recent years worldwide. This is because the local crops have been substituted by genetically uniform varieties with higher yields. Legumes in general and the genus *Vicia* in particular, are not an exception to this problem, and the areas where many *Vicia* species are cultivated have been dramatically reduced.

Vicia faba is the *Vicia* species most frequently grown for human consumption, and various studies have highlighted its nutritional (Elsherbeeny and Robertson 1992; Krause and Schwenke 1996; Cepeda *et al*. 1998; Kushwah *et al*. 2002; El Fiel *et al*. 2003) and functional properties (Okada and Okada 2000).

The nutritional properties of other *Vicia* species, for example, *Vicia sativa,* have also been reported (Alzueta *et al*. 2001; Seabra *et al*. 2001). The polyphenol content and antioxidative activity of *V. faba* (Amarowicz *et al*. 1996, 2004) and *V. sativa* (Amarowicz *et al*. 2008) have also been studied. The present study determined the antioxidative activity of the polyphenolic fraction extracted from the seeds of 28 *Vicia* species collected in Andalusia, Spain. These species are representative of the variability of the genus *Vicia* in the Mediterranean Region, and include both wild and cultivated taxa.

MATERIALS AND METHODS

Chemicals

Butylated hydroxytoluene (BHT) (+)-catechin, β-carotene and linoleic acid are products of Sigma (Madrid, Spain). Tween 20 was purchased from VWR (Barcelona, Spain).

Plant Material

Fully matured seed samples were taken from wild populations located in Andalusia (southern Spain). The seeds were collected from different fruits and specimens in a given population and stored at –20C. Voucher specimens were deposited in the Herbarium at the Department of Plant Biology and Ecology, University of Sevilla. The following *Vicia* species belonging to two different subgenera and nine sections according to Castroviejo and Pascual (1999) were studied:

Subgenus *Vicia*
Section *Vicia*
- *V. angustifolia* L.
- *V. cordata* Hoppe.
- *V. lathyroides* L.
- *V. pyrenaica* Pourr.
- *V. sativa* L.

Subgenus *Vicia*
Section *Hypechusa*
- *V. hybrida* L.
- *V. lutea* subsp. *cavanillesii* (Mart. Mart.) Romero Zarco.
- *V. lutea* subsp. *lutea* var. *lutea* L.
- *V. lutea* subsp. *lutea* var. *hirta* (Balb. ex Lam. & DC.) Loisel.
- *V. lutea* subsp. *vestita* (Boiss.) Rouy.

Subgenus *Vicia*
Section *Peregrina*
- *V. peregrina* L.

Subgenus *Vicia*
Section *Faba*
- *V. faba* L.
- *V. narbonensis* L.

Subgenus *Cracca*
Section *Pedunculata*
- *V. altissima* Desf.
- *V. onobrychioides* L.

Subgenus *Cracca*
Section *Cracca*
- *V. benghalensis* L.
- *V. dasycarpa* Ten.
- *V. disperma* DC.
- *V. eriocarpa* (Hausskn.) Halácsy.
- *V. glauca* C. Presl.
- *V. incana* Gouan.
- *V. monantha* subsp. *calcarata* Retz.
- *V. monardi* Boiss.
- *V. pseudocracca* Bertol.
- *V. tenuifolia* Roth.
- *V. vicioides* (Desf.) Cout.

Subgenus *Cracca*
Section *Ervum*
- *V. hirsuta* (L.) Gray.
- *V. parviflora* Cav.
- *V. pubescens* (DC.) Link.

Subgenus *Cracca*
Section *Ervoides*
- *V. articulata* Hornem.

Subgenus *Cracca*
Section *Ervilia*
- *V. ervilia* (L.) Willd.

Polyphenols Extraction and Quantification

Seeds were ground using a domestic blender and extracted (60 mg) with methanol (1 mL) by vortexing in Eppendorf tubes at maximum speed for 1 h at room temperature in the

dark. The methanolic extracts were recovered by centrifugation at 12,000 rpm for 15 min and stored in the dark at −20°. The total phenolic content of methanolic extracts was determined according to Mazza *et al*. (1999) method. The sample (10 μL) was mixed with a solution of 2% HCl in 75% ethanol (240 μL) in a 96-well microtiter plate. After 10 min, the absorbance of the solution was monitored at 280 nm to measure total phenolics. Catechin dissolved in methanol was used as a standard. Phenolic content was expressed as milligrams equivalent of catechin per gram of sample.

Antioxidative Activity

Antioxidant activity was estimated by determination of the peroxidative decomposition of β-carotene (bleaching) in the presence of linoleic acid and the samples as described by Marco (1968) modified method. A mixture of β-carotene (1 mL, 10 mg/mL in chloroform), linoleic acid (20 mg) and Tween 20 (200 mg) was vortexed and flushed with nitrogen in order to eliminate chloroform. After addition of oxygen-sparged distilled water (50 mL), the mixture was vortexed again in order to obtain a clear solution. Methanolic extracts (5 μL) or polyphenols (2 μg) and β-carotene assay solution (200 μL) were added to the wells of 96-well plates and incubated at 50C. Absorbance (450 nm) was read at 10 min intervals during 1 h. Data on the inhibition of β-carotene bleaching by the samples was processed following different methods as previously described (Oomah and Mazza 1996; Velioglu *et al*. 1998). For the first method, the log of the absorbance was plotted against time, as a kinetic curve, and the slope was expressed as the antioxidant value (AOX).

The second method employed involved the calculation of the degradation rate as previously reported by Al-Saikhan *et al*. (1995) as follows:

$$DR\,(\text{degradation rate}) = Ln\,(\text{Absorbance at 0 min}/\text{Absorbance at 60 min})/60$$

and the antioxidant activity $(AA) = (DR_{\text{control}} - DR_{\text{sample}}) \times 100/DR_{\text{control}}$

The third method of expression, based on the oxidation ratio (Marinova *et al*. 1994) was calculated from the equation:

$$\text{Oxidation rate ratio (ORR)} = DR_{\text{sample}}/DR_{\text{control}}$$

Lastly, the antioxidant activity coefficient (AAC) according to Mallet *et al*. (1994) was calculated as:

$$AAC = (Abs_{\text{sample60}} - Abs_{\text{control60}}) \times 1{,}000/(Abs_{\text{control0}} - Abs_{\text{control60}})$$

where Abs_{sample60} and $Abs_{\text{control60}}$ are the absorbance of samples and controls after 60 min incubations, respectively, and Abs_{control0} is the absorbance of controls at time 0.

RESULTS AND DISCUSSION

Polyphenols in *Vicia* Seeds

The concentration of total polyphenols in the seeds corresponding to 28 *Vicia* species collected from Andalusia, Spain was determined by measuring the absorbance of their methanolic extracts at 280 nm after acidification. All the seeds were collected from wild populations even though they are sometimes cultivated (Table 1). Polyphenol concentrations observed in cultivated *Vicia* species ranged from 1.9 mg/g ground seed in *V. narbonensis* to 21.3 mg/g in *V. sativa*. Polyphenols in noncultivated *Vicia* species ranged from 2.1 mg/g ground seed *in V. lutea* ssp. *lutea* var. *hirta* to 11.6 mg/g in *V. pyrenaica*. Average polyphenol concentration in *Vicia* was 6.0 ± 3.9 mg/g ground seed, as compared to 13.66 ± 9.2 mg/g in the related *Lathyrus* genus (Pastor-Cavada *et al.* 2009). The polyphenol concentration in several commercial grain legumes were found to be within the range as observed in the *Vicia* samples in this study. The polyphenol concentration in chickpea, lupin, and soy were found to be 6.4, 5.3 and 5.3 mg/g ground seed, respectively.

The variability in polyphenol concentration in *Vicia* was high even between taxonomically related species. For instance, polyphenol concentration in *V. lutea* subspecies ranged between 2.1 and 8.5 mg/g of ground seed. The polyphenol concentration did not correlate with the section and subgenus to which the studied *Vicia* belonged. For example, the subgenus *Vicia* includes the sections with the lowest and highest polyphenol concentration. Section *Vicia* with 11.7 ± 5.4 mg polyphenol/g ground seeds include species with high polyphenols contents such as *V. angustifolia* (11.4 mg/g), *V. pyrenaica* (11.6 mg/g) and *V. sativa* (21.3 mg/g). The lowest concentrations were found in section *Peregrina* represented *by V. peregrina* (2.8 mg/g), and section *Faba* (3.3 ± 1.3 mg/g) represented by *V. narbonensis* (1.9 mg/g). In subgenus *Cracca* section *Ervilia*, *V. ervilia* had the highest polyphenol concentration (7.9 mg/g). Section *Cracca* in the same subgenus, which include the highest number of species, had the lowest average polyphenol concentration, 4.0 ± 1.8 mg/g ground seeds.

Antioxidant Activity in *Vicia* Methanolic Extracts

Antioxidant activity in the *Vicia* methanolic extracts was determined by measuring their inhibitory effect on the oxidative degradation of β-carotene in the presence of linoleic acid. In a first series of experiments, this activity was referred to as polyphenol content (specific activity) by taking for the assays the volume of extract corresponding to 2 μg catechin equivalents (Table 1). Specific activity in the *V. parviflora*, *V. tenuifolia*, and *V. lathyroides* extracts were the highest among the

TABLE 1. POLYPHENOLS CONCENTRATION AND ANTIOXIDANT ACTIVITY IN METHANOLIC EXTRACTS (2 μg CATECHIN EQUIVALENTS) FROM *VICIA* SPECIES

	AOX	DR	AA	ORR	AAC	Polyphenol contents*
V. altissima	0.0047	0.0103	56.40	0.44	392.27	4.4
V. angustifolia	0.0052	0.0117	50.72	0.49	332.60	11.4
V. articulata	0.0047	0.0113	52.02	0.48	346.96	6.8
V. benghalensis	0.0036	0.0083	65.17	0.35	482.87	2.7
V. cordata	0.0048	0.0113	52.13	0.48	348.07	9.8
V. dasycarpa	0.0034	0.0074	68.94	0.31	519.34	2.1
V. disperma	0.0033	0.0077	67.51	0.33	509.39	2.8
V. eriocarpa	0.0042	0.0096	59.63	0.40	427.62	3.6
V. ervilia	0.0042	0.0093	60.60	0.40	434.25	7.9
V. faba	0.0062	0.0141	40.62	0.59	244.20	4.6
V. glauca	0.0045	0.0101	57.46	0.43	398.90	6.4
V. hirsuta	0.0044	0.0105	55.65	0.44	382.32	7.4
V. hybrida	0.0056	0.0127	46.48	0.54	300.55	7.3
V. incana	0.0042	0.0094	60.23	0.40	426.52	4.6
V. lathyroides	0.0033	0.0077	67.69	0.32	522.65	4.5
V. lutea subsp. *cavanillesii*	0.0037	0.0081	65.78	0.34	485.08	8.5
V. lutea subsp. *lutea* var. *hirta*	0.0045	0.0102	56.82	0.43	398.90	2.1
V. lutea subsp. *lutea* var. *lutea*	0.0045	0.0108	54.65	0.45	372.38	5.3
V. lutea subsp. *vestita*	0.0048	0.0108	54.59	0.45	371.27	5.5
V. monantha subsp. *calcarata*	0.0045	0.0095	60.07	0.40	429.83	3.8
V. monardi	0.0056	0.0128	45.86	0.54	289.50	5.7
V. narbonensis	0.0048	0.0112	52.67	0.47	346.96	1.9
V. onobrychioides	0.0049	0.0112	52.66	0.47	348.07	8.7
V. parviflora	0.0032	0.0074	68.76	0.31	527.07	3.2
V. peregrina	0.0040	0.0090	62.04	0.38	460.77	2.8
V. pseudocracca	0.0045	0.0103	56.59	0.43	390.06	2.2
V. pubescens	0.0036	0.0087	63.19	0.37	461.88	6.2
V. pyrenaica	0.0049	0.0114	51.78	0.48	344.75	11.6
V. sativa	0.0046	0.0111	53.17	0.47	358.01	21.3
V. tenuifolia	0.0034	0.0077	67.65	0.32	527.07	2.8
V. vicioides	0.0052	0.0116	50.96	0.49	338.12	7.7

Results are the average of three independent determinations.

* mg/g seed flour. Taxa that are marginally cultivated or were cultivated in the past are underlined.

AOX, antioxidant value; DR, degradation rate; AA, antioxidant activity; ORR, oxidation rate ratio; AAC, antioxidant activity coefficient.

extracts from noncultivated *Vicia*, while *V. dasycarpa* yielded the extract with the highest specific activity among cultivated taxa (Table 1). The lowest antioxidant activity among noncultivated and cultivated taxa was found in the extracts from *V. hybrida* and in *V. faba*, respectively. No significant correlation was found between antioxidant activity and the *Vicia* sections and subgenus to which the samples belong. The highest antioxidative activity within subgenus *Vicia* was observed in section *Peregrina* represented by *V. peregrina*. Within subgenus *Cracca*, section *Ervoides* represented by *V. articulata* had the lowest antioxidative activity, while section *Ervum* yielded the highest average antioxidative activity.

In a second assay (Table 2), 5 μL methanolic extract was assayed in order to compare antioxidant activity in different taxa as referred to total extract (total activity), and not to polyphenol-equivalents in the extracts (specific activity) as shown in Table 1. These experiments (Table 2) were carried out because preliminary assays showed that theoretical total activities that were calculated from data on specific activity and the concentration of polyphenol-equivalents in the extracts do not always agree with experimental data. Thus, correlation between polyphenol concentration and antioxidative activity was inconclusive. For instance, the extract from *V. sativa*, which had the highest polyphenol concentration, also showed the highest antioxidative activity, but other taxa with high specific activity and average (e.g., *V. lutea* ssp. *cavanillesii* and *V. ervilia*) or even low (e.g., *V. tenuifolia*) polyphenol concentration (Table 1) also showed high total antioxidative activity (Table 2).

On the other hand, extracts with the lowest polyphenol concentrations also showed lower antioxidant activity, e.g., *V. narbonensis*, *V. lutea* ssp. *lutea* var. *hirta* and *V. dasycarpa*. The *V. sativa* extract showed the highest antioxidant activity and polyphenol concentration of all the cultivated *Vicia*, as

TABLE 2. POLYPHENOLS CONCENTRATION AND ANTIOXIDANT ACTIVITY IN METHANOLIC EXTRACTS (5 μL) FROM *VICIA* SPECIES

	AOX	DR	AA	ORR	AAC	Polyphenol contents*
V. altissima	0.0049	0.0128	42.15	0.58	273.03	4.4
V. angustifolia	0.0042	0.0102	54.03	0.46	377.36	11.4
V. articulata	0.0044	0.0118	46.43	0.54	309.66	6.8
V. benghalensis	0.0046	0.0122	44.98	0.55	294.12	2.7
V. cordata	0.0039	0.0098	55.76	0.44	395.12	9.8
V. dasycarpa	0.0052	0.0133	39.90	0.6	250.83	2.1
V. disperma	0.0046	0.0121	45.44	0.55	304.11	2.8
V. eriocarpa	0.0056	0.0148	32.96	0.67	196.45	3.6
V. ervilia	0.0036	0.0095	57.23	0.43	410.66	7.9
V. faba	0.0062	0.0157	28.77	0.71	165.37	4.6
V. glauca	0.0041	0.0107	51.48	0.49	357.38	6.4
V. hirsuta	0.0045	0.0111	49.66	0.50	338.51	7.4
V. hybrida	0.0053	0.0134	39.55	0.61	247.50	7.3
V. incana	0.0046	0.0119	46.12	0.54	305.22	4.6
V. lathyroides	0.0038	0.0098	55.71	0.44	399.56	4.5
V. lutea subsp. *cavanillesii*	0.0027	0.0073	67.07	0.33	524.97	8.5
V. lutea subsp. *lutea* var. *hirta*	0.0057	0.0154	30.47	0.7	176.47	2.1
V. lutea subsp. *lutea* var. *lutea*	0.005	0.0132	40.07	0.60	255.27	5.3
V. lutea subsp. *vestita*	0.0047	0.0123	44.17	0.56	293.01	5.5
V. monantha subsp. *calcarata*	0.0048	0.0128	42.31	0.58	270.81	3.8
V. monardi	0.0051	0.0137	37.88	0.62	234.18	5.7
V. narbonensis	0.0057	0.0156	29.63	0.70	173.14	1.9
V. onobrychioides	0.0041	0.0106	52.21	0.48	368.48	8.7
V. parviflora	0.0039	0.0105	52.51	0.48	368.48	3.2
V. peregrina	0.0052	0.0134	39.38	0.61	248.61	2.8
V. pseudocracca	0.0056	0.0145	34.22	0.66	208.66	2.2
V. pubescens	0.0037	0.0097	56.20	0.44	399.56	6.2
V. pyrenaica	0.0039	0.0100	54.54	0.46	388.46	11.6
V. sativa	0.0023	0.0059	73.21	0.27	597.11	21.3
V. tenuifolia	0.004	0.0098	55.82	0.44	400.66	2.8
V. vicioides	0.0046	0.0118	46.68	0.53	311.86	7.7

Results are the average of three independent determinations.

* mg/g seed flour. Taxa that are marginally cultivated or were cultivated in the past are underlined.

AOX, antioxidant value; DR, degradation rate; AA, antioxidant activity; ORR, oxidation rate ratio; AAC, antioxidant activity coefficient.

opposed to extracts from *V. faba* and *V. narbonensis* which showed the lowest antioxidant activity probably due to low specific activity and low polyphenol concentration, respectively. Extracts from *V. lutea* ssp. *cavanillesii* and *V. lutea* ssp. *lutea* var. *hirta* showed the highest and lowest antioxidant activity of all the noncultivated taxa.

A positive correlation between polyphenol concentration and antioxidative activity in the methanolic extracts was revealed by plotting these two variables (Fig. 1A, r^2 0.3483). This correlation was higher when considering *Vicia* sections alone (Fig. 1B, r^2 0.6787).

Tables 3 and 4 showed that the *Vicia* extracts had the highest antioxidant activity relative to the extracts of the corresponding grain legumes chickpea, lupin and soy, and the synthetic antioxidant BHT and catechin. Activity was refereed to catechin equivalents in the extracts (specific activity, Table 3) or to extract volume (total activity, Table 4). Both specific and total activity in these *Vicia* extracts at least doubled the activity in the extracts corresponding to the grain legumes, and were similar to the activity of 1.28 μg of BHT and 15 μg of catechin (Tables 3 and 4).

The antioxidant activity of different concentrations of methanolic extracts of *V. parviflora*, *V. lathyroides*, *V. tenuifolia*, *V. dasycarpa*, *V. sativa*, *V. lutea*, chickpea, lupin and soy was investigated (Fig. 2). Results showed a similar trend to saturation of the antioxidative activity with increasing polyphenol concentration starting from about 10 μg catechin equivalents/mL in the *Vicia* extracts. Saturation occurred much earlier in the extracts from lupin and soy at about 2 μg catechin equivalents/well.

In conclusion, field work in southern Spain allowed for collection of 28 *Vicia* which are representative of the variability of this genus in Spain, including both cultivated and noncultivated species. Extraction of the ground seeds using

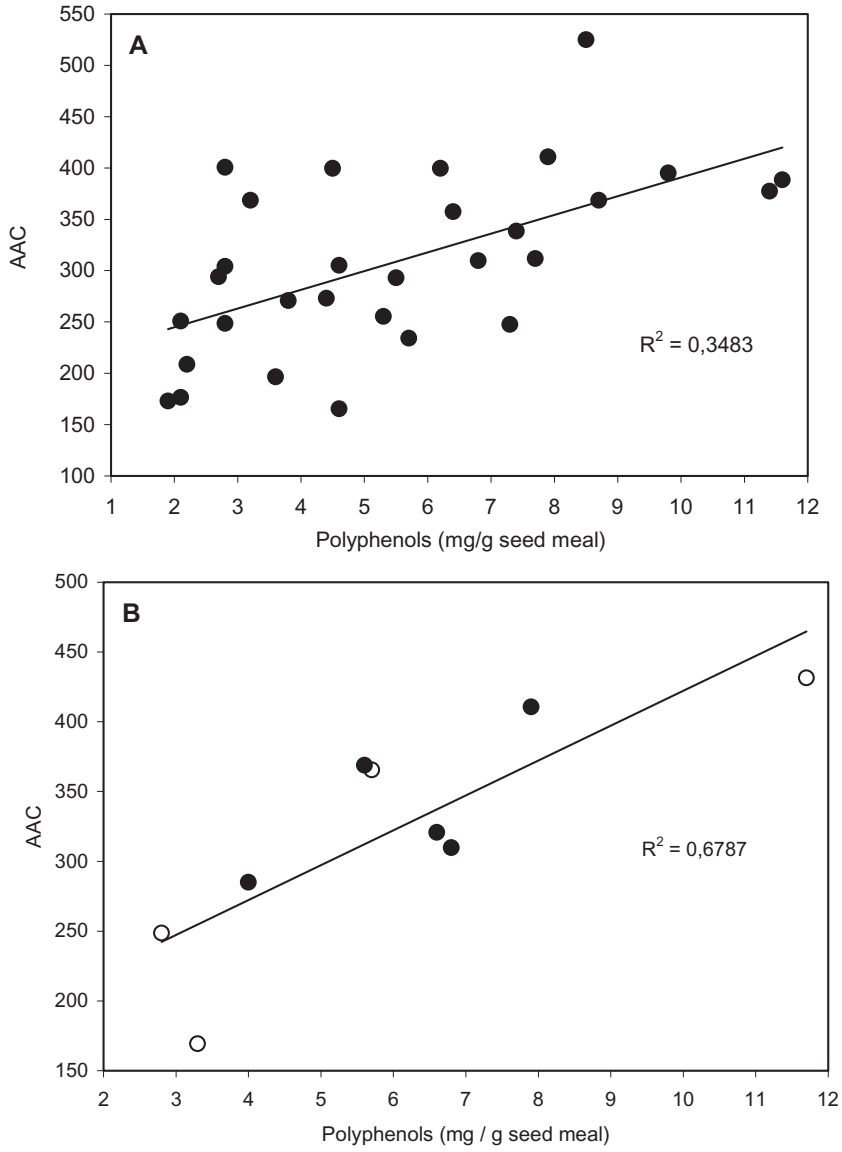

FIG. 1. CORRELATION BETWEEN POLYPHENOL CONCENTRATION (MG CATECHIN-EQUIVALENTS/G GROUND SEED) AND ANTIOXIDANT ACTIVITY COEFFICIENT (AAC) IN METHANOLIC EXTRACTS CORRESPONDING TO *VICIA* SPECIES (A) AND *VICIA* SECTIONS INCLUDING SUBGENUS *VICIA* (B, OPEN CIRCLES) AND SUBGENUS *CRACCA* (B, FULL CIRCLES)

TABLE 3. ANTIOXIDANT ACTIVITY IN METHANOLIC EXTRACTS (2 μg CATECHIN EQUIVALENTS) FROM *VICIA PARVIFLORA, VICIA LATHYROIDES, VICIA TENUIFOLIA, VICIA DASYCARPA*, CHICKPEA, LUPIN, SOY AND DIFFERENT AMOUNTS OF BHT AND CATECHIN

	AV	DR	AA	ORR	AAC
V. parviflora	0.0033	0.0073	59.46	0.405	472.22
V. lathyroides	0.0039	0.0085	52.70	0.473	402.78
V. tenuifolia	0.0032	0.0069	61.63	0.394	513.89
Chickpea	0.0062	0.0137	24.06	0.759	152.78
Lupin	0.0062	0.0140	22.11	0.779	138.89
Soy	0.0056	0.0127	29.67	0.703	194.44
BHT (0.64 μg/well)	0.004	0.0089	50.70	0.493	375.00
BHT (1.28 μg/well)	0.0031	0.0070	61.20	0.388	500.00
BHT (1.92 μg/well)	0.0027	0.0059	66.98	0.330	555.56
Catechin (5 μg/well)	0.0047	0.0104	42.44	0.576	291.67
Catechin (10 μg/well)	0.0039	0.0085	52.99	0.470	388.89
Catechin (15 μg/well)	0.0034	0.0076	57.66	0.423	444.44

Results are the average of three independent determinations.
AV, antioxidant value; DR, degradation rate; AA, antioxidant activity; ORR, oxidation rate ratio; AAC, antioxidant activity coefficient.

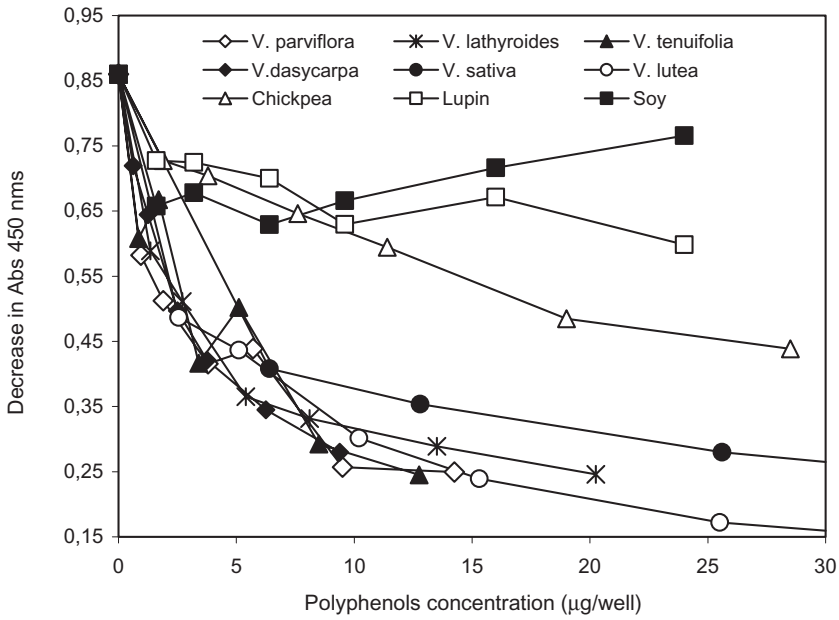

FIG. 2. KINETIC OF ANTIOXIDANT ACTIVITY WITH INCREASING AMOUNTS OF METHANOLIC EXTRACTS OF *VICIA PARVIFLORA*, *VICIA LATHYROIDES*, *VICIA TENUIFOLIA*, *VICIA DASYCARPA*, *VICIA SATIVA*, *VICIA LUTEA*, CHICKPEA, LUPIN AND SOY. RESULTS ARE THE AVERAGE OF THREE INDEPENDENT DETERMINATIONS

methanol yielded preparations with variable polyphenol concentrations and antioxidant activities. The antioxidant activity in most of the extracts corresponding to the *Vicia* species was higher than in the extracts of corresponding chickpea, lupin and soy, and was similar to the antioxidant activity of the synthetic antioxidant BHT. Therefore, *Vicia* may represent a useful source of polyphenols with high antioxidant activity. Polyphenols are plant secondary metabolites with alleged health-promoting properties. The health-promoting effects of a diet rich in legumes have been partly attributed to polyphenols. This study highlights the potential of *Vicia* species, marginally cultivated nowadays, as a source of polyphenols as functional components for foods.

TABLE 4. ANTIOXIDANT ACTIVITY IN METHANOLIC EXTRACTS (5 µL) FROM *VICIA SATIVA* AND *VICIA LUTEA* SSP. *CAVANILLESII*, CHICKPEA, LUPIN AND SOY AND DIFFERENT AMOUNTS OF BHT AND CATECHIN

	AV	DR	AA	ORR	AAC
V. sativa	0.0023	0.0055	66.53	0.335	528.10
V. lutea subsp. *cavanillesii*	0.0029	0.0068	58.53	0.415	444.96
Chickpea	0.0054	0.0124	24.02	0.760	138.17
Lupin	0.0053	0.0121	25.22	0.748	151.05
Soy	0.0046	0.0105	35.51	0.645	227.17
BHT (0.64 µg/well)	0.004	0.0089	50.70	0.493	375.00
BHT (1.28 µg/well)	0.0031	0.0070	61.20	0.388	500.00
BHT (1.92 µg/well)	0.0027	0.0059	66.98	0.330	555.56
Catechin (5 µg/well)	0.0047	0.0104	42.44	0.576	291.67
Catechin (10 µg/well)	0.0039	0.0085	52.99	0.470	388.89
Catechin (15 µg/well)	0.0034	0.0076	57.66	0.423	444.44

Results are the average of three independent determinations.
AV, antioxidant value; DR, degradation rate; AA, antioxidant activity; ORR, oxidation rate ratio; AAC, antioxidant activity coefficient.

ACKNOWLEDGMENT

This work was financed with grant AGR-711 from Junta de Andalucía, Andalucía (Spain).

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