Original article

Wild blackthorn (*Prunus spinosa* L.) and hawthorn (*Crataegus monogyna* Jacq.) fruits as valuable sources of antioxidants

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Wild blackthorn (*Prunus spinosa* L.) and hawthorn (*Crataegus monogyna* Jacq.) fruits as valuable sources of antioxidants.

Abstract – Introduction. Many underutilized wild fruits have great nutritional and functional potential, providing chemical compounds with biological properties. **Materials and methods**. In the present work we quantified bioactive compounds such as vitamin C (ascorbic and dehydroascorbic acids), and total phenolic compounds composed mainly of phenolic acids, flavonols and anthocyanins, as well as evaluating the antioxidant capacity through different *in vitro* tests (Folin-Ciocalteu, $ABTS^{\bullet+}$, $DPPH^{\bullet}$ and FRAP) in wild blackthorn (*P. spinosa* L.) and hawthorn (*C. monogyna* Jacq.) fruits of Spanish origin, including samples from different seasons and locations. **Results and discussion**. As expected, wide variability was found in the composition of fruits of the same species, which justifies the necessity of analyzing several batches of wild fruits, in order to have representative results taking into account the natural variability. Fruits of *P. spinosa* showed vitamin C content ranging between (5.14 and 15.35) mg·100 g^{-1} fw (mainly dehydroascorbic acid); total phenolic compounds ranged from (1851 to 3825) mg·100 g^{-1} fw, characterized by a high content of anthocyanins and phenolic acids. Fruits of *C. monogyna* presented (16 to 39) mg vitamin C·100 g^{-1} fw and (449 to 1438) mg total phenolic compounds·100 g^{-1} fw, characterized by a high content of phenolic acids and flavonols. Antioxidant capacity was higher for *P. spinosa* fruits than for *C. monogyna* fruits; $DPPH^{\bullet}$ values showed a strong correlation with vitamin C, while phenolic compounds were a major contributor to the antioxidant activity of these fruit extracts. Fruits of *P. spinosa* and *C. monogyna* should be reconsidered as new valuable sources of safe and inexpensive antioxidants.

Spain / *Prunus spinosa* / *Crataegus monogyna* / fruits / antioxidants / ascorbic acid / phenolic compounds / flavonoids / anthocyanins

Les fruits du prunellier sauvage (*Prunus spinosa* L.) et de l'aubépine (*Crataegus monogyna* Jacq.) sont de précieuses sources d'antioxydants.

Résumé – Introduction. Beaucoup de fruits d'espèces sauvages sous-utilisées ont un grand potentiel nutritionnel et fonctionnel car ils offrent des composés chimiques présentant des propriétés biologiques. **Matériel et méthodes**. Dans notre travail, nous avons quantifié les composés bioactifs tels que la vitamine C (acide ascorbique et acide déhydroascorbique), les composés totaux phénoliques constitués principalement par des acides phénoliques, des flavonols et des anthocyanines, et nous avons évalué la capacité antioxydante à l'aide de différents tests *in vitro* (Folin-Ciocalteu, $ABTS^{\bullet+}$, $DPPH^{\bullet}$, et FRAP) dans des fruits du prunellier sauvage (*P. spinosa* L.) et de l'aubépine (*C. monogyna* Jacq.) d'origine espagnole, à partir d'échantillons de différentes saisons et lieux. **Résultats et discussion**. Comme prévu, la composition des fruits d'une même espèce a présenté une grande variabilité ce qui a justifié d'analyser plusieurs lots de fruits sauvages, afin de disposer de résultats représentatifs tenant compte de la variabilité naturelle. Les fruits de *P. spinosa* ont montré une teneur en vitamine C comprise entre (5.14 et 15.35) mg·100 g^{-1} mf (principalement constituée d'acide déhydroascorbique) ; les composés phénoliques totaux ont varié de (1851 à 3825) mg·100 g^{-1} mf ; ils ont été caractérisés par une haute teneur en anthocyanes et en acides phénoliques. Les fruits de *M. crataegus* ont présenté de (16 à 39) mg de vitamine C·100 g^{-1} mf et de (449 à 1438) mg de composés phénoliques totaux·100 g^{-1} mf, caractérisés par une haute teneur en acides phénoliques et en flavonoïdes. La capacité antioxydante a été plus élevée pour les fruits de *P. spinosa* que pour ceux de *C. monogyna* ; l'activité antioxydante du test $DPPH^{\bullet}$ a montré une forte corrélation avec la teneur en vitamine C, tandis que les composés phénoliques ont été un contributeur majeur de l'activité antioxydante des extraits de fruits étudiés. Les fruits de *P. spinosa* et *C. monogyna* devraient être mieux considérés en tant que nouvelles sources d'antioxydants sûrs et peu coûteux.

Espagne / *Prunus spinosa* / *Crataegus monogyna* / fruits / antioxydant / acide ascorbique / composé phénolique / flavonoïde / anthocyane

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1. Introduction

Food antioxidants are useful tools to prevent the negative effects of free radicals in the human body, which may lead to a reduction of the risk of some chronic diseases related to the redox state of the human body [1, 2]. The food industry has widely used antioxidants to extend the shelf life of food products. Currently, natural antioxidants, due to their limited sources and high price, are not widely used. Synthetic antioxidants are commonly used in the food industry; nevertheless, the recovery of new sources of safe and inexpensive antioxidants of natural origin could be a good strategy for the food and pharmaceutical industries to replace synthetic antioxidants, avoiding potential health risks and toxicity [3].

Traditionally, people all over the Mediterranean consume a diversity of plants, which are often gathered from the wild, and have remained particularly important when normal food supply mechanisms are disrupted, or when local or displaced populations have limited access to other types of foods [4]. For that reason, nowadays, there is an emerging interest in the international community in consuming many underutilized wild food plants, with a linkage between agriculture, nutrition and health [5]. Their nutritional role and health uses have been reported in many nutritional and ethnobotanical studies worldwide [6, 7]. Mediterranean wild fruits could also be considered as interesting high-value nutraceuticals, being a source of antioxidants for dietary supplements or functional foods [8, 9], as is the case of unusual wild fruits, such as those of *Prunus spinosa* L. and *Crataegus monogyna* Jacq., which may have potential as a source of bioactive compounds with antioxidant activity.

Prunus spinosa (Rosaceae), also known as blackthorn, is a deciduous shrub native to Europe, western Asia and northwest Africa. Its astringent fruits are sometimes consumed overripe, but much more usually processed into jams, or macerated with sugar, honey and liquor to obtain a digestive liqueur used for its laxative, astringent, diuretic and purgative properties [10]. Some authors reported a moderate antioxidant capacity of *P. spinosa* fruits from Poland, which are very popular either raw or processed [11].

Crataegus monogyna (Rosaceae), also known as hawthorn, are small trees and shrubs naturally growing in Europe, Asia and the north of Africa. Flowers, leaves and fruits from *C. monogyna* are known for medicinal use, especially against cardiovascular disease, and have also been used in folk medicine as a cure for stress, nervousness, sleep disorders, stomach ache and sore throat [12]. Both the fruits and flowers of hawthorns are well known in herbal folk medicine as a heart tonic. Hawthorn is combined with ginkgo (*Ginkgo biloba*) to enhance poor memory, by improving the blood supply to the brain. The fruit is also antispasmodic, diuretic and sedative [12]. Some flower and fruit constituents responsible for free radical scavenging activity are epicatechin, hyperoside and chlorogenic acid [13].

To our knowledge, the scientific literature available about anthocyanin and flavonol distribution in these wild fruits is scarce, especially in the case of *P. spinosa*. They could be valuable potential sources of safe and inexpensive antioxidants of natural origin, which requires reconsideration of their role in traditional as well as contemporary/modern diets. Therefore, it seems important to provide information on the bioactive compound contents and their antioxidant capacity, in order to promote and recover their consumption. Our work was focused on the evaluation of wild blackthorn and hawthorn fruits as potential sources of bioactive compounds (vitamin C as ascorbic acid and dehydroascorbic acid, total phenolic compounds, and the profile of phenolic families), as well as the evaluation of their antioxidant capacity measured by different *in vitro* methods.

2. Materials and methods

2.1. Plant material and sample preparation

Wild blackthorn (*Prunus spinosa*) and hawthorn (*Crataegus monogyna*) fruits

were collected from two different sites in the province of Madrid, located in the central area of Spain (*figure 1*), during three consecutive seasons (2007, 2008 and 2009). The species were clearly identified following the descriptions and keys of the two genera included in the work *Flora Ibérica* [14, 15]. Fruits were gathered in their optimal ripening status (November-December) from different trees, randomly selected in both natural forest locations. Each sample consisted of at least 500 g of fruits. All the selected wild fruit presented a healthy external appearance. Samples were packed in plastic recipients and carried to the laboratories in cool conditions within the day. Upon arrival at the laboratory, stems and/or leaves were removed, and fruits were deseeded. Fresh samples were used to evaluate dry matter content. Subsamples of fruits were freeze-dried (Telstar-Cryodos Lyophilizer, Telstar Industrial, Tarrasa, Spain) at –45 °C under vacuum, keeping the samples protected from light throughout the process. The lyophilized product obtained was crushed and homogenized, and stored in the dark in sealed polyethylene bottles at –22 °C until analysis. Three replicates were extracted and measured for each analytical trial.

***Prunus spinosa* L.**

***Crataegus monogyna* Jacq.**

	Site 1	Site 2
Prunus spinosa		
Municipality	El Cuadron	Rascafria
Province	Madrid	Madrid
Coordinates	3°39' W; 40°56' N	3°54' W; 40°52' N
Altitude	1120 m	1163 m
Prunus spinosa		
Municipality	Tielmes	Cantoblanco-Madrid
Province	Madrid	Madrid
Coordinates	3°18' W; 40° 14' N	3°41' W; 40°32' N
Altitude	594 m	690 m

Figure 1.
Details of the collecting sites in the central aera of Spain for the fruits of *Prunus spinosa* L. and *Crataegus monogyna* Jacq. analyzed.

2.2. Dry matter determination

Dry matter was determined by desiccation of fresh fruits to constant weight at (100 ± 2) °C following AOAC procedures [16].

2.3. Vitamin C analysis

The HPLC method proposed by Sánchez-Mata *et al.* [17] for vitamin C, ascorbic acid and dehydroascorbic acid determination was conducted, through the extraction of homogenized freeze-dried fruits in 4.5% *m*-phosphoric acid and HPLC analysis of ascorbic acid; an aliquot was reduced with 4% L-cysteine, at pH 7 for HPLC analysis of total vitamin C in the form of ascorbic acid. Chromatographic conditions involved a Sphereclone ODS (2) (250 mm × 4.60 mm, 5 µm) Phenomenex column, isocratic 1.8 mM H_2SO_4 as the mobile phase and UV detection at 245 nm. The HPLC equipment was a Micron Analitica (Jasco-Spain, Madrid, Spain) chromatographer, and data were analyzed using Biocrom XP software. Quantification was performed by comparison of areas with those obtained with ascorbic acid commercial standard solutions. Dehydroascorbic acid was determined by the difference between total vitamin C (measured in reduced extracts) and ascorbic acid contents. Results were expressed as mg ascorbic acid·100 g^{-1} of fresh weight.

2.4. Phenolic acid, flavonol and anthocyanin HPLC analysis

An aliquot of 0.5 g of freeze-dried fruits was extracted with 20 mL of acidic (0.01 M formic acid) methanol/water (50:50, pH 2). The extract was centrifuged at 1935 *g* for 15 min and the supernatant was recovered. Then, twenty mL of acetone/water (70:30) was added to the residue, and the tubes were shaken and centrifuged again. Methanol and acetone extracts were combined and used to determine phenolic compounds and antioxidant activity in the samples [18]. HPLC analysis of phenolic compounds was performed using a C18 Hypersil ODS

stainless steel column (250 mm × 4.6 mm, 5 µm) (Teknokroma, Barcelona, Spain) thermostated at 30 °C. The equipment consisted of an Agilent 1100 Series System equipped with a quaternary pump, autosampler system and rapid scanning UV-visible photodiode array detector. The solvent system used was a gradient of acetonitrile (solvent A) and formic acid 2% (solvent B), as follows: 0 min, 4% of solvent A; 10 min, 10% of solvent A; 20 min, 20% of solvent A; 30 min, 40% of solvent A; 35 min, 40% of solvent A; 40 min, 60% of solvent A; 45 min, 60% of solvent A; 55 min, 4% of solvent A. The flow rate was 1 mL·min^{-1} and runs were monitored with the UV-visible photodiode array detector set at 280 nm (phenolic acids), 360 nm (flavonols) and 520 nm (anthocyanins). Data were processed using an Agilent ChemStation software. Identification of the main phenolic compounds was carried out by comparing the retention times with those of the standards and by comparing with chromatographic data found in the literature. The phenolic acids (λ 280 nm) were quantified as mg gallic acid Eq·100 g^{-1} of fresh weight, the flavonols (λ 360 nm) were quantified as mg rutin Eq·100 g^{-1} of fresh weight, and the anthocyanins (λ 520 nm) were quantified as mg pelargonidin 3-glucoside Eq·100 g^{-1} of fresh weight. The quantification was carried out using external standard calibration curves (gallic acid, rutin and pelargonidin 3-glucoside) ranging between 50 µg·mL^{-1} and 300 µg·mL^{-1}. Total phenolic compounds were the sum of the three phenolic families expressed as total phenolic compounds per 100 g of fresh weight.

2.5. Antioxidant activity determination

Previously obtained extracts (described in section 2.4) were used for all the *in vitro* antioxidant activity assays.

2.5.1. Folin-Ciocalteu method

Although the Folin-Ciocalteu assay has been traditionally used as a method to determine total phenol content in many plant foods, this reagent can also measure the total reducing capacity of a sample. This method is based on an electron transfer reaction such as the ferric ion reducing antioxidant power (FRAP) assay, among others. This is the reason why this trial is being used for antioxidant capacity determination [19], providing information complementary to phenolic compound content and other antioxidant assays. In our work, aliquots of 0.5 mL of methanol/water extracts were introduced into test tubes; another 0.5 mL of Folin-Ciocalteu reagent and 10 mL of sodium carbonate (7.5%) were added, and flasks were made up to 50 mL with distilled water [20]. After 60 min in the dark, absorbance was measured at 750 nm in a Lambda Ez 210 UV-visible spectrophotometer (Perkin Elmer, Massachusetts, USA). Results were compared with a standard curve prepared daily with different concentrations of gallic acid and expressed as mg gallic acid Eq·100 g^{-1} of fresh weight.

2.5.2. 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) radical cation ($ABTS^{\cdot+}$) scavenging capacity assay

The $ABTS^{\cdot+}$ assay is a decolorization assay applicable to both lipophilic and hydrophilic antioxidants. The pre-formed radical monocation of 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{\cdot+}$) is generated by oxidation of ABTS with potassium persulfate and reduced in the presence of hydrogen-donating antioxidants, according to the method of Re *et al.* [21], with some modifications. The ABTS radical cation ($ABTS^{\cdot+}$) was produced by the reaction of ABTS with 2.45 mM potassium persulfate ($K_2S_2O_8$), allowing the mixture to stand in the dark at room temperature for 12–16 h before use. The $ABTS^{\cdot+}$ solution (stable for two days) was diluted with ethanol to an absorbance of 0.70 ± 0.02 at 734 nm. Then, ten µL of each extract were incorporated into a 96-well microplate, and 290 µL of 7 mM $ABTS^{\cdot+}$ were added, mixed well, and, after 20 min in the dark at 30 °C, absorbance was measured at 734 nm. Results were compared with a standard curve prepared daily with different concentrations of trolox and expressed as mmol Trolox Eq·100 g^{-1} of fresh weight.

2.5.3. 2,2´-Diphenyl-1-picrylhydrazyl radical (DPPH$^{\bullet}$) scavenging capacity assay

The DPPH$^{\bullet}$ radical is a stable radical widely used to monitor the free radical scavenging abilities of various antioxidants, through the loss of absorbance at 515 nm as the pale yellow non-radical form is produced. The method proposed by Sánchez-Moreno *et al.* [22], with some modifications, was followed. Briefly, ten µL of each extract were mixed with 290 µL of 100 µM DPPH$^{\bullet}$ in methanol in a 96-cell microplate, and, after one hour of incubation in the dark, absorbance was measured at 515 nm in a microplate reader. Results were compared with a standard curve prepared daily with different concentrations of trolox and expressed as mmol of Trolox Eq·100 g^{-1} of fresh weight.

2.5.4. Ferric reducing antioxidant power assay

In the ferric reducing antioxidant power (FRAP) assay, a potential antioxidant reduces ferric ion (Fe^{3+}) to ferrous ion (Fe^{2+}) at low pH, with the formation of a blue complex (Fe^{2+}/TPTZ) [23]. The ferric ion reducing antioxidant power reagent was freshly prepared by mixing together 0.3 M acetate buffer (pH 3.6), ten mM TPTZ (2,4,6-tris-2,4,6-tripyridyl-2-triazine) in 40 mM HCl and 20 mM $FeCl_3$ in the proportion 10:1:1 (v/v/v), respectively. The assay was carried out in a 96-well microplate, by adding 10 µL of each extract and 290 µL of the FRAP reagent. After 20 min shaking in the dark at 37 °C, absorbance was measured at 593 nm. Results were compared with a standard curve prepared daily with different concentrations of trolox and expressed as mmol Trolox Eq·100 g^{-1} of fresh weight.

2.6. Statistical analysis

All the analyses were carried out in triplicate. Statgraphics Plus 5.1 (Statistical Graphics Corporation, Inc., Rockville, MD, USA) was used for statistical treatment of the analytical data. The multivariate ANOVA test and Fisher's Least Significant Difference (LSD) *post hoc* test were used to compare pairs of means and determine statistical significance at the $P < 0.05$ level. The correlations within variables were examined by Pearson correlation. Also, Principal Component Analysis (PCA) was performed among the variables analyzed using Statgraphics Plus 5.1 software.

3. Results and discussion

3.1. Vitamin C and phenolic compounds of the fruits

In agreement with previous studies of other fruits [8, 24, 25], geographical, seasonal and ripening status variations were expected to influence the chemical composition of the fruits as a result of differences in soil composition, sun exposition and climate; this expectation was observed from the results of our study. These variations justified the necessity of analyzing several batches of wild fruits, from different sites and years of collection, in order to take into account this natural variability in the final results. In general, we can consider that our results were coherent with those reported by other authors [26, 27].

The fruits of *P. spinosa* had much less total vitamin C than *C. monogyna*, with average values of (11.27 and 30.35) mg·100 g^{-1} fw, respectively (*table I*). In the former, ascorbic acid contributes only 1.33% to the total content of vitamin C, which means that the major vitamin C content in the fruits of *P. spinosa* was dehydroascorbic acid. In previous studies, it could be seen that the vitamin C content of the blackthorn fruits was slightly higher than that of the present data, 21.94 mg·100 g^{-1} fw [10]. *Crataegus monogyna* fruits were also poor in ascorbic acid, with dehydroascorbic acid contributing to 92.9% of the vitamin C content [(16.01–39.40) mg·100 g^{-1} fw] in the fruits of *C. monogyna* (*table I*). As in both fruits ascorbic acid (the reduced form of vitamin C) is found in very low amounts, this may suggest a slight contribution of vitamin C to the antioxidant capacity of these fruits.

Table I.
Vitamin C (ascorbic and dehydroascorbic acids), phenolic acid, flavonol, anthocyanin and total phenolic compounds of *Prunus spinosa* L. and *Crataegus monogyna* Jacq. fruits.

Prunus spinosa L. (blackthorn)

Year and site	Moisture (%)	Total vitamin C	Ascorbic acid	Dehydroascorbic acid	Phenolic acids[1] (mg gallic acid Eq·100 g^{-1} fw)	Flavonols[2] (mg rutin Eq·100 g^{-1} fw)	Anthocyanins[3] (mg pelargonidin 3-glucoside Eq·100 g^{-1} fw)	Total phenolic compounds (mg·100 g^{-1} fw)
		(mg·100 g^{-1} fw)						
2007								
Site 1	66.92 ± 1.49 ab	9.85 ± 0.54 c	0.34 ± 0.01 b	9.50 ± 0.54 b	823.00 ± 65.68 e	116.12 ± 9.94 c	1239.04 ± 78.08 d	2178.16 ± 20.05 e
Site 2	65.23 ± 1.63 a	13.89 ± 0.18 e	0.08 ± 0.00 a	13.81 ± 0.19 d	802.07 ± 54.98 d	141.86 ± 10.06 e	1188.01 ± 65.01 c	2131.94 ± 16.79 d
2008								
Site 1	67.64 ± 0.42 bc	15.35 ± 0.99 f	0.41 ± 0.08 c	15.10 ± 1.03 e	985.56 ± 34.76 f	226.69 ± 15.76 f	2585.32 ± 151.67 f	3797.57 ± 30.07
Site 2	72.43 ± 0.45 d	12.36 ± 0.21 d	Not detected	12.36 ± 0.21 c	757.02 ± 49.75 c	100.95 ± 9.23 b	1128.57 ± 84.09 a	1980.53 ± 15.96
2009								
Site 1	67.08 ± 0.32 b	8.75 ± 0.23 b	Not detected	8.75 ± 0.23 b	574.84 ± 60.86 b	130.82 ± 10.40 d	1288.56 ± 84.96 e	1994.23 ± 20.14 c
Site 2	68.76 ± 0.20 c	5.14 ± 0.29 a	Traces	5.14 ± 0.29 a	430.38 ± 32.02 a	87.63 ± 6.45 a	1160.98 ± 71.65 b	1678.99 ± 14.58
Average	68.33	11.27	0.26	11.18	728.81	134.01	1431.75	2294.57

Crataegus monogyna Jacq. (hawthorn)

Year and site	Moisture (%)	Total vitamin C	Ascorbic acid	Dehydroascorbic acid	Phenolic acids[1] (mg gallic acid Eq·100 g^{-1} fw)	Flavonols[2] (mg rutin Eq·100 g^{-1} fw)	Anthocyanins[3] (mg pelargonidin 3-glucoside Eq·100 g^{-1} fw)	Total phenolic compounds (mg·100 g^{-1} fw)
		(mg·100 g^{-1} fw)						
2007								
Site 1	56.93 ± 4.21 a	39.40 ± 0.77 d	0.58 ± 0.05 a	39.01 ± 0.65 e	879.03 ± 32.45 f	447.68 ± 21.98 f	47.32 ± 2.68 f	1374.04 ± 1.40
Site 2	80.78 ± 7.89 c	16.01 ± 0.15 a	0.58 ± 0.07 a	15.34 ± 0.20 a	242.00 ± 11.89 a	77.18 ± 1.79 b	27.81 ± 1.64 d	346.99 ± 4.35 a
2008								
Site 1	70.20 ± 1.06 b	27.79 ± 1.39 b	1.29 ± 0.13 b	25.97 ± 1.28 b	362.66 ± 16.24 c	174.01 ± 3.78 d	10.66 ± 1.02 a	547.32 ± 4.75 b
Site 2	73.23 ± 0.60 b	32.38 ± 0.49 c	5.01 ± 0.26 e	27.65 ± 0.94 b	267.17 ± 13.45 b	267.35 ± 4.76 e	28.46 ± 1.87 e	562.97 ± 4.75
2009								
Site 1	70.18 ± 0.68 b	34.22 ± 3.06 c	2.65 ± 0.18 c	31.85 ± 2.57 c	549.23 ± 40.02 e	96.06 ± 2.59 c	24.60 ± 2.02 b	669.89 ± 7.45 e
Site 2	67.69 ± 0.33 b	37.79 ± 2.65 d	3.25 ± 0.02 d	34.85 ± 2.19 d	499.02 ± 30.74 d	60.88 ± 1.94 a	24.74 ± 1.65 c	584.63 ± 8.45 d
Average	69.84	30.35	2.15	28.28	466.52	187.19	27.26	680.98

Values expressed as mean ± standard deviation (SD), $n = 3$. In each column, different letters mean statistically significant differences ($P < 0.05$).

[1] determined by HPLC ($\lambda = 280$ nm); [2] determined by HPLC ($\lambda = 360$ nm); [3] determined by HPLC ($\lambda = 520$ nm).

The fruits of *P. spinosa* were richer sources of phenolic compounds than the fruits of *C. monogyna*, with the fractions of anthocyanins and phenolic acids as the major ones (*figure 2*). For *P. spinosa*, anthocyanins ranged between (1128.6 and 2585.3) mg·100 g^{-1} fw (*table I*), with a contribution to the total phenolic compounds of 62.4%, and phenolic acids ranged between (430.38 and 985.56) mg·100 g^{-1} of fresh fruit, contributing 31.76%. Flavonols were the minor family in *P. spinosa*, ranging between (87.6 and 226.7) mg·100 g^{-1} fw (*table I*). Phenolic compound families presented a different profile in *C. monogyna,* where phenolic acids were predominant, with a contribution to the total phenolic compounds of 68.5% [(267.2 and 879.0) mg·100 g^{-1} fw], followed by 27.5% flavonols [(60.88 and 447.68) mg·100 g^{-1} fw], and 4% anthocyanins [(10.66–47.32) mg·100 g^{-1} fw] (*table I*).

3.2. Identification of phenolic compounds of the fruits

The identification of phenolic acid compounds (280 nm), flavonols (360 nm) and anthocyanins (520 nm) in *P. spinosa* and *C. monogyna* fruits was performed by comparing retention times and spectral data with authentic standards and scientific published data of similar fruits.

The HPLC chromatogram of *P. spinosa* fruits at 280 nm presented only one main compound, which was identified as gallic acid by comparison with the authentic standard; caffeic acid was also tentatively identified (*table II*). At 360 nm, quercetin 3-glycoside was identified. At 520 nm, cyanidin 3-rutinoside, cyanidin 3-glucoside and peonidin 3-glucoside were identified according to their retention times and absorption maxima in the UV-Vis spectra (*table II*).

In the case of *C. monogyna* fruits, the major compound identified at 280 nm was gallic acid, and chlorogenic acid and epicatechin were also tentatively identified. Also, at 360 nm, the HPLC profile showed two main compounds which chromatographic and spectral data matched with quercetin 3,4-diglucoside and quercetin 3,7,4-triglucoside. At 520 nm, cyanidin 3-galactoside was identified according to its retention time and absorption maxima in the UV-Vis spectra (*table II*).

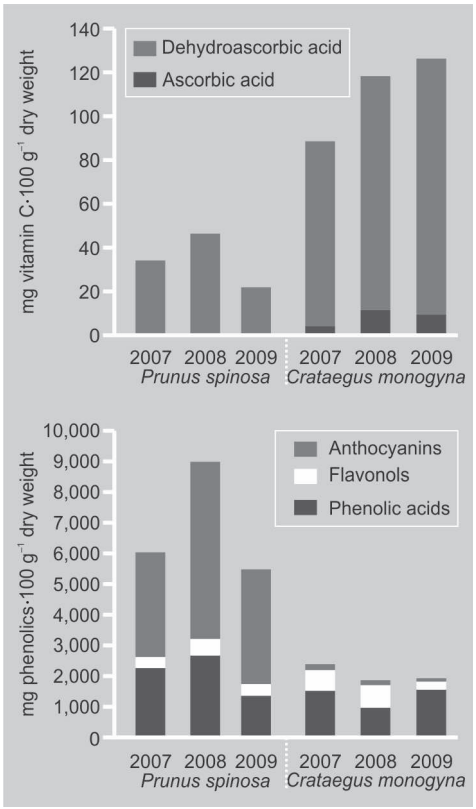

Figure 2.
Distribution of different antioxidant compounds [vitamin C (ascorbic acid and dehydroascorbic acid) and phenolics] in *Prunus spinosa* and *Crataegus monogyna* fruits (average of each year of harvest, expressed as dry weight).

Previous studies have shown that the mean dietary intake of total phenolic compounds is 780 mg per day for females and 1058 mg per day for males, with half of these composed of hydroxycinnamates, 20–25% of total flavonoids, and approximately 1% of anthocyanins [28]. These requirements can be achieved with the intake of portions of aproximately 50 g of *P. spinosa* and 150 g of *C. monogyna* fruits (edible part).

3.3. Antioxidant capacity of the fruits

Prunus spinosa, which showed the highest phenolic compound content, was also found to have the highest antioxidant capacity by the Folin-Ciocalteu method, and $ABTS^{\bullet+}$ and FRAP methods (*table III*). In our study, *Prunus spinosa* analyzed by the Folin-Ciocalteu method gave results ranging

Table II.
Chromatographic and spectroscopic characteristics, and tentative identification of the three main groups of phenolic compounds identified (phenolic acids, flavonols and anthocyanins) in *Prunus spinosa* L. and *Crataegus monogyna* Jacq. fruits.

Prunus spinosa L. (blackthorn)				
Detection λ (nm)	Retention time (min)	Compound	% peak area	λ_{max} (nm)
280	4.71	Gallic acid	73.59	265
	24.15	Caffeic acid	2.74	280
360	10.92	Quercetin 3-glycoside	66.39	325
520	27.06	Cyanidin 3-rutinoside	69.80	520
	29.26	Cyanidin 3-glucoside	22.22	520
	29.88	Peonidin 3-glucoside	7.97	520
Crataegus monogyna Jacq. (hawthorn)				
Detection λ (nm)	Retention time (min)	Compound	% peak area	λ_{max} (nm)
280	4.96	gallic acid	34.44	265
	17.76	chlorogenic acid	5.15	280
	18.84	epicatechin	13.32	280
360	25.66	quercetin 3,4-diglucoside	51.56	355
	25.89	quercetin 3,7,4-triglucoside	19.20	355
520	26.75	cyanidin 3-galactoside	100	520

from (1851.93 to 3825.93) mg gallic acid Eq·100 g^{-1} of fresh fruit. The $ABTS^{\bullet+}$ method gave results from (1.83 to 7.64) mM Trolox Eq·100 g^{-1} of fresh fruit. These values are in accordance with those of Ganhao *et al.*, who reported values of 5.51 mM Trolox Eq·100 g^{-1} for these fruits [26]. However, Jablonska-Rys *et al.* gave a lower value for the Folin-Ciocalteu method (402.67 mg gallic acid Eq·100 g^{-1}), and $ABTS^{\bullet+}$ and FRAP methods [10]. The FRAP method values ranged from (7.11 to 15.17) mmol Trolox Eq·100 g^{-1} of fresh fruit.

The *Crataegus monogyna* fruits analyzed in our study showed values of (449.38 to 1438.52) mg gallic acid Eq·100 g^{-1} for Folin-Ciocalteu determination (*table III*), higher values than those reported by Ganhao *et al.* [26] and Egea *et al.* [29] [(450 and 216.61) mg gallic acid Eq·100 g^{-1}, respectively]. Applying the $ABTS^{\bullet+}$ method, the values for fruits of *C. monogyna* ranged from (1.68 to 6.12) mmol Trolox Eq·100 g^{-1} of fresh fruit, which matches Ganhao *et al.*, who provided 5.68 mM Trolox Eq·100 g^{-1} of fresh fruit [26], and are in accordance with the results of Froehlicher *et al.* [30]. The values obtained by the FRAP method ranged from (3.28 to 10.99) mmol Trolox Eq·100 g^{-1} of fresh fruit.

3.4. Correlations of antioxidant activity values and phenolic compounds

As the $ABTS^{\bullet+}$ and FRAP methods reflect a similar trend of antioxidant capacity in the samples analyzed, these *in vitro* tests could be a good choice to characterize the antioxidant capacity of phenolic-rich fruits. Only $DPPH^{\bullet}$ showed a strong correlation with total vitamin C (r = 0.7099, $P < 0.05$) and its major form in the analyzed fruits,

Table III.
Antioxidant capacity of *Prunus spinosa* L. and *Crataegus monogyna* Jacq. fruits ($ABTS^{\bullet+}$: 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) radical cation; $DPPH^{\bullet}$: 2,2´-Diphenyl-1-picrylhydrazyl radical; FRAP: ferric reducing antioxidant power).

Prunus spinosa L. (blackthorn)				
Year and site	Folin-Ciocalteu (mg of gallic acid Eq·100 g^{-1} fw)	$ABTS^{\bullet+}$	$DPPH^{\bullet}$	FRAP
		(mmol of Trolox Eq·100 g^{-1} fw)		
2007				
Site 1	2188.60 ± 152.61 bc	2.77 ± 0.23 b	0.92 ± 0.07 a	7.11 ± 0.38 a
Site 2	2307.71 ± 66.67 c	5.75 ± 0.50 c	1.02 ± 0.08 a	9.90 ± 0.33 c
2008				
Site 1	3825.93 ± 164.82 d	7.64 ± 0.74 d	1.39 ± 0.08 c	13.04 ± 0.38 d
Site 2	1983.54 ± 124.13 ab	6.14 ± 0.24 c	0.96 ± 0.03 a	9.28 ± 0.04 b
2009				
Site 1	1990.90 ± 72.96 ab	5.64 ± 0.35 c	1.37 ± 0.04 c	15.17 ± 0.21e
Site 2	1851.93 ± 147.08 a	1.83 ± 0.15 a	1.17 ± 0.04 b	10.35 ± 0.47 c
Average	2255.57	5.08	1.14	10.81
Crataegus monogyna Jacq. (hawthorn)				
Year and site	Folin-Ciocalteu (mg of gallic acid Eq·100 g^{-1} fw)	$ABTS^{\bullet+}$	$DPPH^{\bullet}$	FRAP
		(mmol of Trolox Eq·100 g^{-1} fw)		
2007				
Site 1	1438.52 ± 66.98 d	4.55 ± 0.45 c	2.03 ± 0.17 d	8.66 ± 0.37 c
Site 2	449.38 ± 21.54 a	1.75 ± 0.17 a	0.76 ± 0.05 a	3.28 ± 0.05 a
2008				
Site 1	583.88 ± 36.91 b	1.68 ± 0.13 a	1.01 ± 0.02 ab	3.55 ± 0.17 a
Site 2	823.20 ± 15.18 c	3.85 ± 0.32 b	1.27 ± 0.12 b	5.20 ± 0.38 b
2009				
Site 1	807.72 ± 20.80 c	4.34 ± 0.06 c	1.80 ± 0.13 c	8.51 ± 0.49 c
Site 2	628.61 ± 15.44 b	6.12 ± 0.11 d	1.84 ± 0.08 cd	10.99 ± 0.66 d
Average	820.55	3.77	1.54	7.11

Values expressed as mean ± standard deviation (SD), $n = 3$. In each column, different letters mean statistically significant differences ($P < 0.05$).

dehydroascorbic acid ($r = 0.7248$, $P < 0.05$) (*table IV*). On the other hand, we could affirm that phenolic compounds were a major contributor to the antioxidant activity of these fruit extracts. This last assertion was confirmed by the strong correlations found between total phenolic compounds (quantified by HPLC) and antioxidant activities by the Folin-Ciocalteu ($r = 0.9939$, $P < 0.05$), $ABTS^{\bullet+}$ ($r = 0.6147$, $P < 0.05$) and FRAP ($r = 0.6863$, $P < 0.05$) methods. Also, strong correlations were found between phenolic acids and anthocyanins with antioxidant activities by Folin-Ciocalteu ($r = 0.7970$ and $r = 0.9603$, $P < 0.05$), $ABTS^{\bullet+}$ ($r = 0.7163$ and $r = 0.4969$, $P < 0.05$) and FRAP ($r = 0.6863$

Table IV.
Correlation analysis of antioxidant activity values with total vitamin C, ascorbic acid, dehydroascorbic acid, total phenols and the three main groups of phenolic compounds identified (phenolic acids, flavonols and anthocyanins) in fruits of *Prunus spinosa* and *Crataegus monogyna* ($ABTS^{\bullet+}$: 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) radical cation; $DPPH^{\bullet}$: 2,2´-Diphenyl-1-picrylhydrazyl radical; FRAP: ferric reducing antioxidant power).

Compounds	Folin-Ciocalteu		$ABTS^{\bullet+}$		$DPPH^{\bullet}$		FRAP	
	r	*P*	*r*	*P*	*r*	*P*	*r*	*P*
Total vitamin C	– 0.5689	0.0003	0.1069	0.5347	0.7099	0.0000	– 0.3064	0.0691
Ascorbic acid	– 0.5543	0.0005	– 0.0650	0.7064	0.3693	0.0267	– 0.4102	0.0130
Dehydroascorbic acid	– 0.5439	0.0006	0.1266	0.4618	0.7248	0.0000	– 0.2768	0.1022
Total phenolic compounds	0.9939	0.0000	0.6147	0.0001	0.3275	0.0512	0.6863	0.0000
Phenolic acids	0.7970	0.0000	0.7163	0.0000	0.2073	0.2250	0.7954	0.0000
Flavonols	0.1292	0.4526	0.2411	0.1566	0.4216	0.0104	0.1699	0.3219
Anthocyanins	0.9603	0.0000	0.4969	0.0020	– 0.2556	0.1325	0.7976	0.0000

r, Pearson correlation coefficient; *P*, p-value.

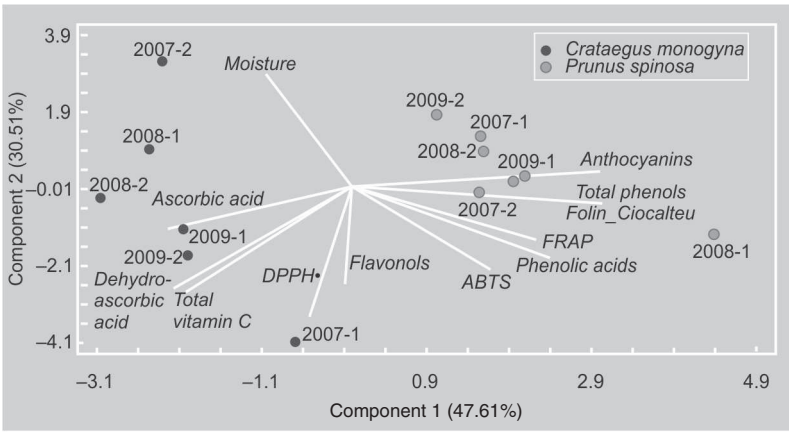

Figure 3.
Principal Component Analysis (PCA) of bioactive compounds of *Prunus spinosa* L. and *Crataegus monogyna* Jacq. fruits. Data from two different sites in the province of Madrid, central area of Spain, during three consecutive seasons (2007, 2008 and 2009).

and $r = 0.7976$, $P < 0.05$) (*table IV*). These correlations should be further studied in the context of the complex synergistic and antagonistic actions of the different bioactive compounds involved in the antioxidant metabolism of plants.

3.5. Classification of the fruits according to their bioactive compounds

Due to the number of variables studied and the variability observed in all of them, multivariate analysis was applied, in order to characterize and classify the fruits studied according to their bioactive compounds. A Principal Component Analysis (PCA) was performed, reducing the multidimensional structure of the data and providing a two-dimensional map to explain the variance observed.

The first two components of the PCA explained 78.12% of the total variance (47.61% for component 1 and 30.51% for component 2) (*figure 3*). All the samples analyzed were plotted on the reduced space of the two principal components, and correlation coefficients were obtained. The first component is highly positively correlated with phenolic compounds (total phenolic compounds, anthocyanins and phenolic acid variables) and antioxidant activity measured by Folin-Ciocalteu, FRAP and $ABTS^{\bullet+}$ assays. It is also negatively correlated with vitamin C and its fractions (ascorbic acid and dehydroascorbic acid). The second principal component separates the samples according to moisture (positive correlation), and it is negatively correlated with dehydroascorbic acid, vitamin C, flavonols, and also correlated negatively with antioxidant activity measured by the $DPPH^{\bullet}$ assay.

Prunus spinosa L. fruits were positively characterized by the first and second principal components (high total phenolic compounds, anthocyanins, and phenolic acids and low vitamin C) and *Crataegus monogyna* negatively correlated with both (high dehydroascorbic acid, vitamin C and flavonols), which statistically confirmed the observations in the data presented.

4. Conclusion

Prunus spinosa fruits (blackthorn) were characterized by a high content of anthocyanins (1431.75 mg pelargonidin 3-glucoside Eq·100 g^{-1} fresh weight) and phenolic acids (728.81 mg gallic acid Eq·100 g^{-1} fresh weight), and low vitamin C levels (11.27 mg ascorbic acid·100^{-1} fresh weight). *Prunus spinosa* fruits had a higher total phenolic compound content (2294.57 mg·100 g^{-1} fresh weight) than *C. monogyna* (680.98 mg·100^{-1} fresh weight) and this fact was related to the more powerful antioxidant activity measured by Folin-Ciocalteu, $ABTS^{\bullet+}$ and FRAP assays.

Crataegus monogyna fruits (hawthorn) showed higher levels of vitamin C (as dehydroascorbic acid) (30.35 mg ascorbic acid·100^{-1} fresh weight) and higher antioxidant activity measured by the DPPH assay than *P. spinosa*. Phenolic acids (466.52 mg GAE·100^{-1} fresh weight) and flavonols (187.19 mg rutin Eq·100^{-1} fresh weight) were the major phenolic families in *C. monogyna*.

Significant strong correlations were found between individual and total phenolic compounds with antioxidant capacity measured by Folin-Ciocalteu, FRAP and $ABTS^{\bullet+}$ assays, indicating that phenolic compounds are the main contributor to the antioxidant capacity of blackthorn and hawthorn fruits.

These results showed that *P. spinosa* and *C. monogyna* fruits are promising sources of natural antioxidants and other bioactive compounds for the food or pharmaceutical industries.

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El fruto del endrino (*Prunus spinosa* L.) y el majuelo (*Crataegus monogyna* Jacq.) constituyen valiosas fuentes de antioxidantes.

Resumen – Introducción. Muchas plantas silvestres tienen un gran potencial nutricional y funcional, proporcionando compuestos químicos con actividades biológicas. **Material y métodos**. En este trabajo, hemos cuantificado compuestos bioactivos como la vitamina C (ácidos ascórbico y dehidroascórbico), compuestos fenólicos totales principalmente ácidos fenólicos, flavonoles y antocianinas, así como evaluado la capacidad antioxidante a través de ensayos *in vitro* (Folin-Ciocalteu, $ABTS^{\bullet+}$, $DPPH^{\bullet}$, and FRAP) en frutos de endrino (*P. spinosa* L.) y majuelo (*C. monogyna* Jacq.) de origen español, incluyendo muestras de diferentes estaciones y localidades. **Resultados y discusión**. Como se esperaba, se encontró una amplia variabilidad en la composición de los frutos de la misma especie, lo que justifica la necesidad de analizar diferentes lotes de frutos silvestres para poder tener en cuenta la variabilidad natural. Los frutos de *P. spinosa* mostraron un contenido de vitamina C que osciló entre (5.14 y 15.35) mg·100 g^{-1} peso fresco; los compuestos fenólicos oscilaron en (1851 a 3825) mg·100 g^{-1} peso fresco, caracterizados por un alto contenido de antocianinas y ácidos fenólicos. Los frutos de *C. monogyna* presentaron (16 a 39) mg vitamin C·100 g^{-1} peso fresco y (449 a 1438) mg de compuestos fenólicos totales·100 g^{-1} peso fresco, caracterizados por un alto contenido de ácidos fenólicos y flavonoles. La capacidad antioxidante fue mayor para los frutos de *P. spinosa* que para los de *C. monogyna*; los valores obtenidos por el método del $DPPH^{\bullet}$ mostraron una fuerte correlación con la vitamina C, mientras que los compuestos fenólicos fueron los que más contribuyeron a la actividad antioxidante de los extractos de estos frutos. Los frutos de *P. spinosa* y *C. monogyna* deberían ser reconsiderados como nuevas y valiosas fuentes de antioxidantes seguros y de bajo coste.

España / *Prunus spinosa* / *Crataegus monogyna* / frutas / antioxidantes / ácido ascórbico / compuestos fenólicos / flavonoides / antocianinas