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ORIGINAL PAPER

Six Edible Wild Fruits as Potential Antioxidant Additives or Nutritional Supplements

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Abstract Total antioxidant activity of six non-cultivated but traditionally collected fruits from the south of Europe was assessed by measuring their ability to reduce the hydroxyl radical ($OH^{\bullet}$) and hydrogen peroxide (H_2O_2), and their Trolox equivalent antioxidant capacity (TEAC). This antioxidant activity was compared with that shown by the synthetic antioxidants BHA (E-320), BHT (E-321) and propyl gallate (E-310). Total phenolics, ascorbic acid and the carotenoid content of the fruits were also analyzed. All fruits showed a high ability to scavenge the $OH^{\bullet}$ radical, ranging from 60.61% to 81.04% inhibition for *Rosa canina* and *Crataegus monogyna*, respectively. The H_2O_2 scavenging capacity and the TEAC value varied widely, ranging between 3.63% and 87.26% inhibition of H_2O_2 and between 0.47 and 416.64 mM trolox g^{-1} FW for *Sorbus domestica* and *Rosa canina*, respectively. The antioxidant activity of fruits was higher than that of the synthetic additives analyzed, except in the TEAC assay. The phenolic and carotenoid content of *R. canina* was much higher than that of the other fruits analyzed and its ascorbic acid concentration was also high, reflecting its higher efficacy towards $ABTS^{\bullet-}$ (TEAC assay) and H_2O_2 species. In spite of these associations, the correlation coefficients between total antioxidant activity and the antioxidant compounds analyzed were not very significant; only phenolics and carotenoids showed a marginal correlation with the TEAC assay. The results support the possible use of *R. canina* as natural antioxidant to replace the synthetic additives, as well as their use in the production of functional foods with a high antioxidant activity.

Keywords Antioxidant activity · Non-cultivated fruit · Radical scavenging capacity · Phytonutrients · Synthetic antioxidant · Functional foods

Introduction

A great variety of non-cultivated plants have long formed part of diet of the Mediterranean region. However, traditions are susceptible to change very quickly and, severed authors suggested that many such habits are at risk of disappearing. It must be understood that there is a close relation between cultural and biological diversities and that a new social, cultural or economic value must be given to local resources if traditions are to be maintained for future generations [1]. The above mentioned plant foods primarily include leaves, fruits, flowers, and seeds of spontaneous trees and shrubs. For this study, we have selected some wild fruits traditionally harvested in autumn from the south of Europe concerning their nutritional properties. In particular, it would be advantageous to assess the antioxidant properties of these plants for possible use in the elaboration of functional foods or for consideration as potential sources of natural antioxidants.

In recent years, there has been growing interest in functional foods, i.e., foods that can provide not only basic nutritional and energetic requirements, but also an addi-

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tional physiological benefit [2, 3]. Usually, the functionality of a food is related to some of its ingredients and consumers increasingly prefer ingredients of a natural origin (i.e., non-synthetic), which can be extracted from plants, food by-products, and other natural sources. Synthetic antioxidants have been suspected of causing or promoting negative health effects [4], and stronger restrictions have been placed on their application, leading to substitute them with naturally occurring antioxidants. As a result, many plant species have been investigated in the search for novel antioxidants. Some natural antioxidants (i.e. rosemary and sage) are already commercially exploited as antioxidant additives or as nutritional supplements [5]. Indeed, among functional ingredients, the most widely studied group is the family of antioxidants. The consumption of fruits with a high antioxidant composition has been associated with a lowered incidence of degenerative diseases including cancer, heart disease, inflammation, arthritis, immune system decline, brain dysfunction, and cataracts [6]. When the level of reactive oxygen species (ROS) exceeds the antioxidant capacity of the cell, the intracellular redox homeostasis is altered, and the resulting oxidative stress may destroy all major classes of biomolecules in the vicinity of their source, including lipids, protein and DNA, a fact closely linked to the ageing processes of tissues and the appearance of diseases [7]. Antioxidants, which can inhibit or delay the oxidation of an oxidisable substrate in a chain reaction would therefore seem to be very important in the prevention of these diseases [6].

The main antioxidants found in fruit are phenolic compounds, ascorbate and carotenoids [6]. Besides the phenolic acids and their derivatives, the fruits always contain members from one or more groups of flavonoids, such as glycosylated flavones/flavonols, flavanones, anthocyanins, proanthocyanidins, as the main phenolic components [8]. As regards the chemical diversity of the antioxidant compounds present in foods and the interactions occurring among their different molecules, the evaluation of the total antioxidant capacity seems to be a more useful marker than the evaluation of a single compound [9]. However, no single method to test the total antioxidant capacity of foods fully considers, at the same time, the activity of all antioxidant compounds. This capacity could vary with the structure of the oxidizing radical, the nature of the substrate for oxidation, the presence of interacting components, the mode of initiating oxidation and even with the analytical method used for measuring oxidation [10]. A possible approach could be to consider several antiradical activities together with the antioxidant compounds present.

The aim of this paper was to study the antiradical activity of six non-cultivated autumn fruits in relation to their phenolic, ascorbic acid and carotenoids content: azarole hawthorn (*Crataegus azarolus* L.), common hawthorn (*Crataegus monogyna* Jacq.), blackthorn (*Prunus spinosa* L.), dog rose (*Rosa canina* L.), blackberry (*Rubus ulmifolius* Schott), and service tree (*Sorbus domestica* L.), all traditionally harvested in the south of Europe. We also compared their antioxidant activities with that found in synthetic antioxidants such as butylated hydroxyanisole (BHA, E-320), butylated hydroxytoluene (BHT, E-321), and propyl gallate (PG, E-310). The findings from this work will be helpful for understanding these fruits and may well be significant for industrial development.

Material and Methods

Plant Material The non-cultivated and traditionally collected autumn fruits of six species from the family of Rosaceae were studied: azarole hawthorn (*Crataegus azarolus* L.), common hawthorn (*Crataegus monogyna* Jacq.), blackthorn (*Prunus spinosa* L.), dog rose (*Rosa canina* L.), blackberry (*Rubus ulmifolius* Schott), and service tree (*Sorbus domestica* L.). Fruits were harvested in different areas of Albacete province (Spain). The physico-chemical characteristics of the fruits are shown in Table 1. The edible part of the fruits was frozen in liquid nitrogen, lyophilized and powdered using a domestic mixer and, finally stored at 20 °C until further analysis.

Sample Extract Preparation Samples were prepared according to Serrano et al. [11] The lyophilized material (0.2 g) was extracted for 1 h by stirring at 4 °C in darkness with 40 mL of phosphate buffer (10 mM KH_2PO_4-KOH buffer, pH 7.4), and centrifuged at 4,500*g* for 30 min. Aliquots of the supernatants were used as fruit extract in the different antioxidant assays. BHA, BHT, and propyl gallate were prepared at the commercial concentration of 100 μg g^{-1}, in the same phosphate buffer [12].

Determination of Antiradical Activity

Hydroxyl Radical ($OH^{\bullet}$) *Scavenging Potential Assay* In a final volume of 1.2 mL the reaction mixtures contained the following reagents: 10 mM KH_2PO_4-KOH buffer (pH 7.4), 2.8 mM H_2O_2, 2.8 mM deoxyribose (DR), 50 μM $FeCl_3$ pre-mixed with 100 μM EDTA before addition to the reaction mixture, and 100 μL of sample. Ascorbate (100 μM) was added to start the reaction. The tubes were incubated at 37 °C for 1 h. The products of the hydroxyl radical ($OH^{\bullet}$) attack on sugar were measured as described in Egea et al. [13] at 532 nm. The results are expressed as percentage inhibition of the DR attack, where 100% attack is defined as the absorbance levels recorded for DR without

Table 1 Physico-chemical caracteristics of six non-cultivated traditionally collected fruits from the south of Europe

Fruit name	TSS[z] (% sucrose)	TA[y] (% malic acid)	Water content[x] (%)	Color[w]			Visual color[v]
				a*	b*	L*	
Rosa canina	77.00±0.05 d	5.94±0.38 d	16.36±1.37 a	34.72±2.83 b	23.69±2.92 c	38.02±1.89 b	Red
Crataegus monogyna	28.50±2.00 c	0.53±0.01 a	73.49±0.35 e	44.12±2.41 c	19.26±2.80 c	34.91±2.12 c	Red
Rubus ulmifolius	13.33±1.53 a	0.55±0.08 a	70.03±0.91 c,d	1.88±0.73 a	1.27±0.39 a	16.37±1.07 a	Black
Prunus espinosa	26.25±1.06 b,c	2.18±0.18 c	66.13±0.18 b	1.36±0.44 a	11.19±1.65 b	38.04±1.95 b	Violet
Crataegus azarolus	24.80±0.72 b	1.24±0.06 b	71.08±0.45 d	5.23±1.41 a	57.74±2.04 d	77.09±1.22 b	Yellow
Sorbus domestica	24.03±1.31 b	0.85±0.06 a,b	68.10±0.18 c	5.54±0.99 a	4.29±3.75 a	35.13±3.09 b	Brown
Significance	*	*	*	*	*	*	*

[z] TSS (Total soluble solids) was determined according with Egea et al. [13]. Values are the mean ± standard deviation (n=3 extracts)

[y] TA (Tritable acidity) were detemined according with Egea et al. [13]. Values are the mean ± standard deviation (n=3 extracts)

[x] The water content was calculates by weight difference between fresh and liophylized fruit

[w] Color of fruits were determined as L*a*b* color space by reflection using a Minolta CR-300 colorimeter. Values are the mean ± standard deviation (n=75 fruits)

[v] Visual color was determined by a trained panel of nine trained judges

Significantly different at $P<0.05$ (a<b<c<d<e)

the addition of the samples. The same reaction without ASC was used to determine whether fruits could reduce hydroxyl radical generation by avoiding the oxidation of ascorbate rather than directly scavenging hydroxyl radicals.

Peroxyl Radical (H_2O_2) Scavenging Potential Assay An aliquot of sample (100 μL) was incubated with 0.84 mM H_2O_2 for 10 min at 25 °C. Aliquots of these mixtures were then taken and assayed for remaining H_2O_2 by using the peroxidase system described by Murcia et al. [12] The remaining H_2O_2 was measured as the formation of a chromophore recorded at 436 nm in reaction mixtures containing, in a final volume of 1 mL, 0.15 M KH_2PO_4-KOH buffer, pH 7.4, 0.5 mM guayacol, and 10 μL of Sigma type IV horseradish peroxidase. The results are expressed as percentage of disappearance of the H_2O_2 of the reaction medium.

Trolox Equivalent Antioxidant Capacity (TEAC Assay) The $ABTS^{\bullet-}$ radical anion solution was generated by incubating, at 60 °C for 6 min, a mixture of 2.5 mM 2,2′-azobis(2-amidinopropane) hydrochloride (ABAP) and 20 mM 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonate) $\left(ABTS_2^-\right)$ stock solution in 100 mL of phosphate buffer (100 mM phosphate and 150 mM NaCl, pH 7.4). The absorbance at 734 nm was measured to check $ABTS^{\bullet-}$ formation [13]. 40 μL of sample were mixed with 1,960 μL of the radical solution to measure the antioxidant activity at 734 nm for a period of 6 min. The decrease in absorbance at 734 nm observed 6 min after the addition of each compound was used to calculate the Trolox equivalent antioxidant capacity (TEAC). A calibration curve was prepared with different concentrations of Trolox (water-soluble analogous to vitamin E). The TEAC activity was calculated according to Egea et al. [13] and represents the concentration of Trolox, in μM, that has the same antioxidant capacity as the analyzed sample (Trolox equivalent).

Determination of Antioxidant Compounds

Ascorbic Acid Fruit ascorbate (ASC) content was determined according to Egea et al. [13]. The lyophilized material (0.3 g) was ground in 10 mL of 50 g Kg^{-1} cold metaphosphoric acid. The final solution was stirred continuously for 30 min at 4 °C in darkness, and then centrifuged at 20,000g for 25 min. The supernatant was passed through a C18 column (Sep-pack plus, Waters) and a 0.2 μm filter. The filtered supernatant was used for ascorbic acid determination using HPLC (Shimadzu LC-10Atvp) with a thermostated ion-exchange column (ION-300) at 30 °C and isocratic elution. The absorbance was recorded with a UV/vis detector at 245 nm. A standard curve in the range 10–100 mg kg^{-1} ascorbic acid was used. The results were expressed as mg ascorbic acid per 100 g of fresh weight (FW).

Total Phenolic Compounds Total phenolic compounds were determined according to Singleton et al. [14] using Folin-Ciocalteu reagent. The lyophilized material (0.3 g) was homogenized in a Polytron® (9.500 rpm) with 3 mL phosphate buffer KH_2PO_4-K_2HPO_4 (50 mM, pH=7.8) for 1 min on ice. The homogenate was centrifuged at 5,500g and 4 °C for 25 min and the supernatant was collected as

final extract. An aliquot of each extract (0.1 mL) was diluted with 0.4 mL of phosphate buffer (50 mM, pH=7.8). Folin-Ciocalteu reagent (2.5 mL) was added and the contents of the flask mixed thoroughly. After 8 min, Na_2CO_3 solution (l0 mL, l0%, w/v) was added and then the samples were incubated in a water bath at 50 °C for 5 min. After that, the blue color produced was measured spectrophotometrically at 760 nm. The concentration of the total phenolic compounds in fruit extracts was determined by comparison with the absorbance of analytical grade phenol standard, gallic acid, at different concentrations. Results were expressed as mg gallic acid per 100 g of fresh weight (FW).

Total Carotenoids The total carotenoid content was measured by extraction with acetone-methanol (1:1), followed by transfer to ethyl ether, saponification with a methanolic solution of KOH (20%), and spectrophotometric measurement of the absorbance of the extract in ethyl ether at 450 nm. The results were expressed as mg β-carotene per 100 g of fresh weight [13].

Statistical Analysis The experimental results were expressed as mean ± standard deviation (SD) of three replicates of 25 randomly selected fruits (75 fruits per sample). Statistical analysis was performed by one-way analysis of variance (ANOVA). The results were calculated using the statistical software (SPSS, version 14.0, SPSS Inc., Chicago, USA). Correlations between different parameters were computed as Pearson's correlation coefficient (r) using Excel for Windows 2000. Differences or correlations were considered significant for $P<0.05$.

Results and Discussion

Scavenging Hydroxyl Radical ($OH^{\bullet}$) Capacity

Hydroxyl radicals are known to be the most reactive of all the reduced forms of dioxygen, and are capable of damaging almost every molecule found in living cells [15]. These radicals have the capacity to join the nucleotides in DNA and cause strand breakage, which contributes to carcinogenesis, mutagenesis and cytotoxicity [16]. In addition, this species is considered to be one of the quick initiators of the lipid peroxidation process due to abstraction of hydrogen atoms from unsaturated fatty acids [15]. Hydroxyl radicals can be formed by the Fenton reaction in the presence of H_2O_2 and reduced transition metals such as Fe^{2+}. Ascorbic acid greatly increases the rate of $OH^{\bullet}$ generation by reducing Fe^{3+} and maintaining the supply of Fe^{2+}. To determine whether non-cultivated fruits could reduce hydroxyl radical generation either by avoiding oxidation of ascorbate or by directly scavenging hydroxyl radicals, the effects of fruit extracts on hydroxyl radicals generated by Fe^{3+} were analyzed by determining the degree of deoxyribose degradation. Table 2 shows the inhibition of hydroxyl radicals by extracts of non-cultivated fruits and synthetic antioxidants in presence and in absence of ascorbate in the reaction medium. The results show that all the fruits had a significantly higher antioxidant activity ($P<0.05$) than the synthetic antioxidants analyzed (BHA, BHT and propyl gallate). The two species of the genus *Crataegus* (*C. monogyna* and *C. azarolus*) and *Prunus spinosa* showed the strongest protective action (81.04%, 78.61% and 80.59% inhibition, respectively), significantly ($P<0.05$) higher than the rest of analyzed fruits. *Rosa canina*, *Sorbus domestica* and *Rubus ulmifolius*, with inhibition percentages of 60.60%, 68.29% and 64.64%, respectively, can be considered as moderately powerful antioxidants in this assay comparing with the percentage inhibition of the different spices (anise, cinnamon, ginger, licorice, mint, nutmeg, and vanilla) [12]. When ascorbate was omitted from the reaction, both *Crataegus* species maintained high antioxidant activity, with inhibition percentages of 73.27% (*C. monogyna*) and 75.30% (*C. azarolus*). These findings confirm that the ability of these two fruits to protect deoxyribose is due to their direct capacity to scavenge hydroxyl radicals. The results showed that the extracts have proton-donating ability and could serve as free radical inhibitors or scavengers, acting possibly as primary antioxidants. However, the antioxidant activity of *Sorbus domestica, Prunus spinosa* and *Rubus ulmifolius* decreased by 13.5%, 35.2% and 81.3%, respectively, when ascorbic acid was removed from the reaction medium. This phenomenon implies that deoxyribose degradation was mainly inhibited by avoiding ascorbate oxidation rather than by the direct scavenging of hydroxyl radicals. Finally, *Rosa canina* did not show antioxidant activity when ascorbate was omitted so; this fruit has no capacity for directly scavenging radical $OH^{\bullet}$.

Hydrogen Peroxide Scavenging Capacity

Hydrogen peroxide may be generated *in vivo* by several oxidase enzymes or by activated phagocytes during the killing of bacterial and fungal strains. There is increasing evidence that H_2O_2, either directly or indirectly via its reduction product $OH^{\bullet}$, may act as a messenger molecule in the synthesis and activation of several inflammatory mediators. When the samples scavenge the hydrogen peroxide, the absorption spectrum decreases through the inhibition of peroxidase activity on H_2O_2. The ability of different extracts of non-cultivated fruits and synthetic antioxidants to scavenge H_2O_2 is shown in Table 3. *Rosa*

Table 2 Scavenging radical $OH^{\bullet}$ capacity of six non-cultivated traditionally collected fruits from the south of Europe, compared with that shown by some synthetic additives

Addition to RM	Oxidative attack to DR[z]			
	With ASC		Without ASC	
	(Abs_{532nm})	% Inhibition[y]	(Abs_{532nm})	% Inhibition[y]
None (control)	1.331	–	0.425	–
Crataegus monogyna	0.252	81.04±0.04 a	0.114	73.27±6.35 a
Prunus espinosa	0.258	80.59±1.77 a	0.204	52.25±6.03 c
Crataegus azarolus	0.285	78.61±0.23 a	0.105	75.3±2.10 a
Sorbus domestica	0.422	68.30±2.37 b	0.175	59.05±1.90 bc
Rubus ulmifolius	0.471	64.64±1.48 b	0.375	12.08±1.17 d
Rosa canina	0.524	60.61±4.39 b	0.421	0.24±0.89 e
BHA	0.973	26.91±2.88[x]	0.109	74.32±3.06[x]
BHT	1.119	15.92±3.22[x]	0.241	43.18±1.55[x]
Propyl gallate	1.92	Prooxidant[x]	1.01	Prooxidant[x]
Significance (fruits × additives)		*		*

RM = Reaction Mixture; DR = Deoxyribose; ASC = Ascorbate

– Not showed percentage of inhibition

[z] The values are mean ± standard deviation (n=3). Means with different letter within a column were significantly different at $P<0.05$ (a>b>c>d<e)

[y] Percentage of inhibition due to the extracts at the final concentration of 5 mg/ml

[x] Percentage of inhibition due to the extracts at the final concentration of 100 μg/g

*Significant differences between fruits and additives at ($P<0.05$)

canina, *Crataegus monogyna* and *Rubus ulmifolius* exhibited the highest activity ($P<0.05$) of all the fruits analyzed, with inhibition percentages of 87.5%, 86.39% and 78.21%, respectively. *Crataegus azarolus* and *Sorbus domestica* had a very low capacity for scavenging H_2O_2 (12.89 and 3.62% of inhibition, respectively). None of the different synthetic antioxidants analyzed (BHA, BHT and propyl gallate) showed a capacity for scavenging H_2O_2 and must be considered inefficient in this respect. In this assay, N-acetyl-L-cysteine, with 97.04% hydrogen peroxide scavenging ability ($P<0.05$), was used as positive hydrogen peroxide scavenger control.

Trolox Equivalent Antioxidant Capacity (TEAC)

The generation of $ABTS^{\bullet-}$ radical anions forms the basis of one of the spectrophotometric methods most widely applied to measure the radical scavenging activity of pure substances, aqueous mixtures and beverages. This assay is an excellent tool for determining the antioxidant activity of hydrogen donating compounds and of chain-breaking antioxidants, and expresses their activity in equivalents of Trolox, an α-tocopherol analogue with enhanced water solubility [17]. The TEAC of the extracts of the six non-cultivated fruits analyzed are presented in Table 3. The species *Rosa canina* with 416.64 μmol Trolox g^{-1} FW showed the highest antioxidant capacity; other species of

Table 3 H_2O_2 scavenging and Trolox equivalent antioxidant capacity (TEAC) of six non-cultivated and traditionally collected fruits from the south of Europe, compared with the activity of some synthetic additives

Addition to RM	Scavenging H_2O_2[z] (% Inibition)[y]	TEAC[z] (μM Trolox g^{-1} FW)
Rosa canina	87.26±0.85 a	416.644±16.73 a
Crataegus monogyna	86.39±0.78 a	8.433±0.83 c
Rubus ulmifolius	78.21±1.82 a	75.391±1.95 b
Prunus espinosa	63.75±0.77 b	80.508±6.29 b
Crataegus azarolus	12.90±4.55 c	4.108±0.32 d
Sorbus domestica	3.63±2.29 d	0.466±0.14 e
Propyl gallate	0.51±0.04 [x]	583.12±5.32[x]
BHA	0.03±0.02 [x]	462.33±6.21[x]
BHT	0.14±0.04 [x]	0.35±0.12[x]
Significance (fruits × additives)	*	*

RM = Reaction Mixture

[z] The values are mean ± standard deviation (n=3). Statistical difference between fruits were analyzed by ANOVA ($P<0.05$). Means with the same letter within a column were not significantly different (a>b>c>d<e)

[y] Percentage of inhibition due to the extracts at the final concentration of 5 mg/ml

[x] Trolox equivalent antioxidant capacity of an additve concentration of 100 μg/g

*Significant differences between fruits and additives at ($P<0.05$)

the same genus have shown very high TEAC levels ranging from 457.2 to 626.2 μmol Trolox g^{-1} FW. This extremely high antioxidant capacity has been attributed to the high phytonutrients content [18]. Such high levels of TEAC are found in some fruits or parts of plants employed for medicinal uses [19], and in other non-cultivated fruits such as blackberry, blueberry or strawberry tree [20]. *Rubus ulmifolius* and *Prunus espinosa*, also showed high antioxidant capacity, with values of 75.39 and 80.51 μmol Trolox g^{-1} FW, respectively, although their activity was five times lower than that of *Rosa canina*. Other authors [21] have studied the antioxidant capacity of a number of *Rubus* species and found TEAC levels between 0 and 25.3 μmol Trolox g^{-1} FW, three times lower than those found in our study. These variations in the results could be explained by different factors like the extraction procedure [22], ripening state of the fruits or genotypic and environmental differences [23], although, coinciding with our results, García-Alonso et al. [20] found in an assay with different fruits that the species *Rubus ulmifolius* was among those with the highest antioxidant capacity. Dall'Acqua et al. [24] reported that this species could be considered as a possible new antioxidant ingredient for the neutraceutical or functional-food market. The two species of the genus *Crataegus* (*C. monogyna* and *C. azarolus*) showed a TEAC value of 8.43 and 4.11 μmol Trolox g^{-1} FW, respectively, 50 times lower than *Rosa canina*. Although these activities are not very high, they are similar to those of grapefruits and oranges, and higher than the antioxidant activity of other fruits like tomato, banana, peach, or apple [25]. Finally, the species *Sorbus domestica* showed the lowest levels of TEAC (0.47 μmol Trolox g^{-1} FW). The additives analyzed, propyl gallate and BHA, showed very high TEAC values; only *R. canina* displayed a similar efficacy. However, the capacity of BHT to scavenge $ABTS^{\bullet-}$ radical was practically null and similar to that of *S. domestica*.

Considering the activity shown in the different antioxidant assays, we noted a certain association between the ability of fruits to scavenge $ABTS^{\bullet-}$ and H_2O_2. In fact, *Rosa canina* showed the highest antioxidant activity against both oxidant species, whereas *Crataegus azarolus* and *Sorbus domestica* presented the lowest activity in these antioxidant assays. This association does not occur between the ability to capture the radical $OH^{\bullet}$ and the rest of assays. The differences shown by the same fruit in different antioxidant tests may be due to the different reaction media and to the different chemical natures of the radical species generated [12]. The oxidant species tested in this study have highly different half-lives and reactivities, while H_2O_2 and $ABTS^{\bullet-}$ are stable radicals, and $OH^{\bullet}$ is much more reactive [12]. These facts could explain why the results vary so much for different radicals. The different antioxidant activity of the six fruits may also have been due to their different antioxidant compositions, meaning that the most plentiful antioxidant compounds in fruits, such as phenolic compounds, ascorbic acid and carotenoid compounds, must be analyzed.

Total Phenolic, Ascorbic Acid and Carotenoid Contents

The main antioxidant substances found in fruits and plants include ascorbic acid, carotenoids and phenolic compounds [3]. Their antioxidant properties are mainly due to their electron-rich structure in the form of double bonds and hydroxyl groups close to each other. The network of hydroxyl groups of some phenolic substances can also chelate free metal cations, for example those from copper and iron, which are powerful pro-oxidants in their free form [26]. There were significant differences ($P<0.05$) between the studied fruits (Table 4) as regards the above three antioxidant compounds. The fruit with the highest levels of total phenols (609.19 mg/100 g FW) was *Rosa canina*. Other authors [18] found higher levels of phenolic compounds in other fruits of the genus *Rosa*. The fruits of *Crataegus azarolus*, *Rubus ulmifolius* and *C. monogyna* also showed high levels of phenolic compounds, 379.16,

Table 4 Total phenolics, ascorbic acid and cartenoids of six non-cultivated and traditionally collected fruits from the South of Europe

Addition to RM	Total phenolics[z] (mg gallic acid $100g^{-1}$)	Total carotenoids[z] (mg β-carotene $100g^{-1}$)	Ascorbic acid[z] (mg $100g^{-1}$)
Rosa canina	609.19±69.81 a	18.07±0.89 a	27.49±2.20 ab
Crataegus monogyna	216.61±28.19 c	1.37±0.22 b	25.11±0.10 b
Rubus ulmifolius	297.39±24.87 bc	0.38±0.02 d	29.08±0.24 a
Prunus espinosa	127.33±4.29 d	0.41±0.03 d	30.75±2.43 a
Crataegus azarolus	379.16±11.78 b	0.58±0.06 c	21.99±2.79 b
Sorbus domestica	8.99±0.47 e	0.29±0.08 d	22.65±0.48 b

RM = Reaction Mixture

[z] The values are mean ± standard deviation ($n=3$). Statistical difference between fruits were analyzed by ANOVA ($P<0.05$), Means with different letter within a column were significantly different at $P<0.05$ (a>b>c>d<e)

297.39 and 216.61 mg 100 g^{-1}FW, respectively; similar and even higher values to those found by Proteggente et al. [25] in regularly consumed fruits considered to have a high polyphenol content, such as strawberry (330 mg/100 g FW), red plum (320 mg/100 g FW) or raspberry (228 mg/100 g FW). Also agreeing with our results, Deighton et al. [21], in a study about the antioxidant properties of genus *Rubus*, measured levels of polyphenols similar to those found in our work for the species *Rubus ulmifolius*, which has been recommended to improve nutritional values in germplasm enhancement programs. The fruits of *Prunus spinosa* showed a significantly lower phenolic content ($P<0.05$) than fruits of *Crataegus azarolus*, *Rubus ulmifolius*, with 127.33 mg/100 g FW, although they may also be considered as a good natural source of polyphenols. These levels are similar to those found in other non-cultivated fruits like deerberry [27], and higher than those of orange, red cabbage and broccoli [25]. On the other hand, Marinova et al. [28] found great variability in the polyphenol content of fruits from different cultivated species of the genus *Prunus*, with values ranging from 50.9 mg/100 g FW in peach (*Prunus persica*) to 303.6 mg/100 g FW in plum (*Prunus domestic*). Finally, the species with the lowest levels of phenols was *Sorbus domestica*, with 8.99 mg/100 g FW, which is below the level found in most regularly consumed fruits [25].

Prunus espinosa and *Rubus ulmifolius* showed the highest levels of ascorbic acid, similar to that recorded in *Rosa canina* but significantly higher ($P<0.05$) than in the rest of analyzed fruits. However, the differences between fruits were not very great, the ascorbic acid content of all fruits ranging between 21.99 and 30.75 mg 100 g^{-1} FW in *Crataegus azarolus* and *Prunus espinosa*, respectively. These ascorbic acid concentrations are high and represent 36.7–51.3% of the recommended daily allowance, RDA, indeed they were higher than those of other fruits like black and green grape, plum, apple, cherry, apricot, melon pear, nectarine, star fruit or ciku, although lower than those of banana, lemon, strawberry, orange, or kiwifruit [6].

R. canina, showed a much higher total carotenoid concentration (18.07 mg 100 g^{-1} FW) than the rest of the fruits (Table 4), 100 g of this fruit representing 430.3% of the RDA. The concentration of total carotenoids of the rest of fruits was lower, ranging between 0.29 and 1.37 mg 100 g^{-1} FW for *Sorbus domestica* and *Crataegus monogyna*, respectively, and representing 6.8–32.7 % of the RDA. These values are higher than those found by Dias et al. [29] in frequently consumed fruits like pear (0.008 mg 100 g^{-1}) and apple (0.04 mg 100 g^{-1}), and similar to those found in other fruits considered as a good source of dietary carotenoids like orange (0.374 mg 100 g^{-1}), cherry (0.261 mg 100 g^{-1}) peach (0.700 mg 100 g^{-1}) and apricot (ranging between 1.5 and 3.5 mg 100 g^{-1} FW, depending on maturity state) [13]. The high carotenoid concentration found in *R. canina* in this study agrees with previously reported results [18, 30]. Gao et al. [18] reported an average carotenoid content of 18 mg 100 g^{-1} in 18 fruits of the genus *Rosa*. According to Razungles et al. [30] the total carotenoids in *R. canina* are 22.4 mg 100 g^{-1} and the major carotenoids are lycopene (11.1 mg 100 g^{-1}), β-carotene (7.2 mg 100 g^{-1}) and β-cryptoxanthin (17.5 mg 100 g^{-1}). Hodisan et al. [31] reported a lower content of total carotenoids (7.8 mg 100 g^{-1}).

Relation of Antioxidant Capacity with Phytonutrient Levels

In this study certain associations between the phytonutrient concentration and total antioxidant activity were found. In fact, *R. canina*, which showed much higher phenolic and carotenoid concentrations than those of the rest of the analyzed fruits (609.19 and 18.07 mg 100 g^{-1} FW, respectively) and a high concentration of ascorbic acid (27.49 mg 100 g^{-1} FW), also showed a much higher capacity for scavenging $ABTS^{\bullet-}$ (416.64 μM trolox g^{-1} FW) and H_2O_2 (87.26% of inhibition) than the rest of the fruits. On the other hand, *S. domestica*, which had a much lower phenolic content than the rest of the fruits (8.99 mg 100 g^{-1} FW), a low carotenoid content (0.29 mg 100 g^{-1} FW) and an ascorbic acid content (22.65 mg 100 g^{-1} FW) that cannot be considered low but which was below the values of the rest of analyzed fruits, also showed low efficiency against the oxidant species $ABTS^{\bullet-}$ (0.47 μM trolox g^{-1} FW) and H_2O_2 (3.62% of inhibition). To check these relations, a correlation analysis between each antioxidant assay and each phytonutrient was made and not very high correlation coefficients were obtained (Table 5). Only

Table 5 Correlation matrix showing interrelations of total antioxidant capacity and phytonutrients

	TEAC*	H_2O_2	$OH^{\bullet}$	Phenolics	Ascorbic	Carotenoid
TEAC	1.000					
H_2O_2	0.523	1.000				
$OH^{\bullet}$	−0.656	−0.160	1.000			
Phenolics	0.778^{MS}	0.449	−0.433	1.000		
Ascorbic	0.390	0.715^{MS}	−0.135	0.119	1.000	
Carotenoid	0.798^{MS}	0.411	−0.617	0.795^{MS}	0.172	1.000

*Trolox equivalent antioxidant capacity of an additve concentration of 100 μg/g

^{MS} Marginally significant ($P>0.05$)

phenolics and carotenoids showed a marginal correlation with the TEAC value, but no similar association was found for ascorbic acids. However, ascorbic acid was associated with the H_2O_2 scavenging capacity. Some studies have shown that the activity in the TEAC assay increases with the number of hydroxyl groups of the antioxidant compound, so the antioxidant activity is higher for phenolic substances than for ascorbic acid, in which only one hydroxyl group reacts in the experimental conditions [31]. This relation between total phenols and the antioxidant activity has been found in many fruits [32], which suggest that the phenolic compounds could be responsible for the antioxidant properties of *Rosa canina*. However, few studies have found a significant correlation between carotenoid content and the TEAC value. The synergistic or antagonistic interactions between different components of fruits may be the cause for the lack of correlation in most fruits studied in this work between the values of the antioxidant activity and their phenolic compound content. In fact, the fruits *Crataegus monogyna* and *C. azarolus* have high levels of polyphenols, but not very high TEAC values. *C. azarolus* offers good protection against the $OH^{\bullet}$ radical, while *Rubus ulmifolius* presents good protection against the H_2O_2 radical. In *Prunus espinosa*, with high TEAC levels and a high protective capacity against the H_2O_2 radical but relatively low levels of polyphenols, the ascorbic acid contributes to a greater content to the antioxidant capacity than in other species with higher levels of polyphenols like *Rosa canina*, *C. monogyna*, *C. azarolus* or *Rubus ulmifolius*. As regards our results, other authors e.g. García-Alonso et al. [20] in a study of the antioxidant capacity of 28 fruits did not find a good correlation between the antioxidant capacity and the phenolic compound content of the samples.

In summary, taking into account both total antioxidant activity and the phytonutrient content, the antioxidant potential in decreasing order of the fruits analyzed was: *R. canina* >> *R. ulmifolius* ≥ *C. monogyna* ≥ *P. espinosa* > *C. azarolus* > *S. domestica.* Of these, *Rosa canina* must be highlighted for showing a much higher antioxidant activity and phytonutrient concentration (phenolic and carotenoid) than the rest of the fruits. The antioxidant potential of all the fruits was higher than that of the additives BHA, BHT and propyl gallate, except in the TEAC assays, where BHA and propyl gallate showed the highest activity, and, generally, also higher than the antioxidant potential described in the literature for many cultivated fruits frequently consumed in Europe [3, 6]. Our results support the possible use of the six analyzed fruits as natural antioxidants to replace the synthetic additives, as well as their use in the production of functional foods with a high antioxidant activity. However, in order to obtain greater efficiency in the use of these fruits as natural antioxidants, it is necessary to know the nature of the medium that must be protected because, according to our study, the same fruit may show different antioxidant efficacies, depending on the reaction medium and the oxidizing species it faces. Finally, although there is a certain association between the phytonutrient content and the total antioxidant activity of the fruits, especially between the phenolic and carotenoid contents and the TEAC value, the correlation coefficient found were not significant. This may be due to the complexity of the antioxidant composition of foods, and to the possible synergistic or antagonistic interaction among the different components in a food mixture. Therefore, the total antioxidant activity of fruits and not only individual antioxidant compounds should always be considered. In spite of the vast literature on phenolic compounds in plants, very little is known on the synergistic or antagonistic interactions between them and other compounds, or how these interactions may affect the total antioxidant capacity of fruit.

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