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RESEARCH ARTICLE

Wild vegetables of the Mediterranean area as valuable sources of bioactive compounds

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Abstract The intake of traditionally consumed wild edible species is nowadays receiving renewed attention, due to the recognition of their potential benefits for human health. This paper represents a contribution to the knowledge of the chemical composition of different wild and under-utilized vegetables of the Mediterranean area, concerning their organic acid profile and the distribution of ascorbic and dehydroascorbic acids as vitamin C activity. Fifteen species, belonging to ten botanical families, were selected, analyzing two samples of each one from two different localities of Central Spain. Each species showed a specific organic acids fingerprint. Citric acid was 90% of total organic acids in *Tamus communis;* malic acid was the major one in *Humulus lupulus, Taraxacum obovatum* and *Cichorium intybus,* and oxalic acid was the main organic acid in *Beta maritima, Papaver rhoeas, Silybum marianum, Foeniculum vulgare, Rumex pulcher, Silene vulgaris, Scolymus hispanicus, Rumex papillaris* and *Bryonia dioica.* The distribution of ascorbic and dehydroascorbic acid was highly variable. Mean values for total vitamin C ranged between 1.5 and 79.4 mg/100 g. *Tamus communis*, *R. pulcher, S. vulgaris* and *B. dioica*,

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showed the highest content of AA, and together with *F. vulgare* and *H. lupulus*, the highest total vitamin C content. These results can be useful to complete food composition databases with the inclusion of wild vegetables from the Mediterranean area, contributing to enhance the diversity of the diet as well as its nutritional quality.

Keywords Bioactive compounds · Neglected and underutilized wild species · Nutritional value · Organic acids · Vitamin C

Introduction

About 7,000 plant species across the world are cultivated or harvested for food or medicinal purpose from the wild vegetation (Ghane et al. 2010). Among these neglected and underutilized species there are many wild species that have played a relevant role both to hunter-gatherers and also to small farmers' subsistence (Price 2003; Vazquez-Garcia 2008; Termote et al. 2011). In fact, Bharucha and Pretty (2010), in a comprehensive review about the role of wild food in agricultural systems, suggested that they continue to form a significant proportion of the global food basket. Some of these locally used wild species have even become cultivated plants, proving their importance for agricultural products and therefore they are often included in catalogues of genetic resources (Hammer et al. 1999). Their consumption is usually limited by their restricted harvest periods, the non-existence of marketing chains, and social stigma attached to them (Pardo-de-Santayana et al. 2007). However, many of them may constitute an interesting genetic resource for the development of new food products (Ruiz-Rodríguez et al. 2011). The knowledge about their chemical and nutritional characteristics is of great interest to understand their agro-industrial potential and to stimulate their commercial exploitation and the investments needed, from farms to factories.

In Spain, like in many other European countries, the consumption of wild greens has played an important role in complementing staple agricultural foods (Hadjichambis et al. 2008; Redzic 2006; Tardío et al. 2006; Vardavas et al. 2006). While the use of non cultivated vegetables has decreased with the development of agriculture and global supply chains, some species are still consumed (Tardío 2010). Their contribution to traditional Mediterranean diets has been qualitatively important (Pieroni et al. 2005; Rivera et al. 2007), and their potential health benefits have been recently shown by different studies supported by the European Commission (Heinrich et al. 2006; Hadjichambis et al. 2008). Some studies have previously reported the nutrient composition of several wild vegetables from Mediterranean countries, such as Italy (Salvatore et al. 2005), Greece (Zeghichi et al. 2003), Spain (Guil et al. 1997; Campra-Madrid and Guil-Guerrero 2002) and Portugal (Barros et al. 2010a, b; Martins et al. 2011). However, data about the nutritional composition of many wild vegetables are scarce and their value is often underestimated.

Wild species may have a great potential as a source of unusual colours and flavours, bioactive compounds and also as sources of dietary supplements or functional foods. Moreover, as the evidence suggests that nutritional success is linked to diversification of the food base, it is important to preserve their traditional uses, as an alternative among the variety of vegetables which we already account. For all those reasons the advance of knowledge about their chemical composition is regarded as an important issue in nutritional and phytotherapy research (Salvatore et al. 2005; Ansari et al. 2005).

From all the biomolecules present in plants, organic acids are primary metabolites, involved in several biochemical pathways; the total content of organic acids in plant tissues is higher than in other organisms, due to their important role as photosynthetic intermediates. The profile of organic acids varies depending upon the species, age of the plant and the tissue type (López-Bucio et al. 2000). Although the acidity of vegetal products is mainly due to citric and malic acid, each organic acid confer different impact; even ascorbic acid can infer acid-sour flavour (Oliveira et al. 2008).

Some organic acids may have biological activity, and thus a significant impact on human health. Oxalic acid is a major compound in many leafy vegetables and although it has a quite low toxicity (with 5 g as the minimal lethal dose for an adult), its negative effect in the reduction of the availability of dietary Ca and in the formation of kidney calculus is well known (Guil et al. 1997). On the other hand, other compounds such tartaric, malic, citric or succinic, have shown health benefits as antioxidants due to their ability to chelate metals (Seabra et al. 2006).

From the organic acids present in plants, ascorbic acid (AA) stands out for its vitamin C activity, together with its oxidized form, dehydroascorbic acid (DHAA), but also because it is a potent antioxidant, either in the food or the human body, by the destruction of oxygen free radicals. The nutritional importance of vitamin C as an essential water-soluble vitamin is well established and it has been recently identified as a key nutrient requiring attention (Phillips et al. 2010). Ascorbic acid (AA) is a cofactor in numerous physiological reactions, and also has a high redox potential, alone and coupled to other antioxidants (Bender 2003). Recent interest in the role of dietary antioxidants in general, and of specific food components, requires accurate food composition data to facilitate epidemiological studies related to the intake of vitamin C, and to develop food consumption recommendations.

Fruits and vegetables (including green leafy vegetables) are major food sources of vitamin C (Eitenmiller et al. 2008). However, very often vitamin C studies in food sources report only AA (with an underestimation of the total vitamin C activity) or the global content of vitamin C (with no data about the distribution of both forms). The separate determination of AA and DHAA in food products provides complete information about vitamin C profile, taking into account that the reduced form (AA) should be preferable, due to its antioxidant properties and higher stability in biological tissues (Schaffer et al. 2005; Ferreres et al. 2006, 2006).

As mentioned before, despite the great scientific interest of wild vegetables, the nutritional characterization and organic acids profiles (including such important compounds as AA and DHAA) of many of them have rarely been assessed. Therefore, the aim of the present study is to survey the specific profile of vitamin C (including AA and DHAA contents) and organic acids of fifteen wild vegetables collected in its natural habitat that had not been previously explored. These new data may help to complete Food Composition Databases, and may contribute to promote the use of these neglected and underutilized plant genetic resources.

Materials and methods

Collection of plant material

According to a previous ethnobotanical review of the wild edible plants traditionally consumed in Spain (Tardío et al. 2006), fifteen of the most commonly used vegetables were selected, belonging to ten different families (Table 1). Plants of each species were identified and collected in two localities (A and B) of the centre of Spain, with different environmental conditions, so a total number of thirty independent samples were analysed (Fig. 1). As wild vegetables are gathered before flowering, when they look similar to other species, a deep knowledge about their vegetative stages is needed for a correct identification of the desired species. Since a study on these wild vegetables production was conducted at the same time (Molina 2009), each sample was selected from at least 25 plants randomly chosen, all of them with a healthy external appearance. They were collected in spring of 2007 in the most appropriate moment for consumption, when the plants were big enough and the edible part was still tender, from the middle of March to late May. Table 1 shows the different edible parts analyzed of each species. At least 500 g of edible portion of each sample was gathered, prepared, packed in plastic bags, and transported to the laboratories in a cold system within the day.

Chemical analysis

Fresh vegetables were immediately homogenized in a laboratory blender. Aliquots were taken to analyze dry matter, pH, titratable acidity, organic acids and vitamin C (AA and DHA).

Dry matter (DM) was determined by desiccation to constant weight (Horwitz and Latimer 2005); pH was measured by potentiometer (MicropH-2000, Crison Instrument) over a homogenized sample 1/10 in distilled water (Horwitz and Latimer 2005); titratable acidity (TA) was determined by titration with 0.1 N NaOH until pH 8.1, and expressed as NaOH meq needed for the neutralization of 100 g of sample. Individual organic acids (oxalic, malic, citric and fumaric) and ascorbic acid (AA) were quantified by High-Performance Liquid Chromatography (HPLC) after extraction with 4.5% *m*-phosphoric acid (Prohens et al. 2005). An aliquot of the extracts were also

Table 1 Selected species, edible parts analysed and traditional culinary uses (based on Tardío et al. 2006)

FAMILY/*Species*	English common name	Edible part	Food use
APIACEAE			
Foeniculum vulgare Mill.	Fennel	Young stems with leaves	Raw (snack, salads) or stewed
ASTERACEAE			
Chondrilla juncea L.	Skeleton weed	Basal leaves	Raw (salads) or stewed
Cichorium intybus L.	Chicory	Basal leaves	Raw (salads) or stewed
Scolymus hispanicus L	Golden thistle	Peeled basal leaves	Stewed, seldom raw in salads
Silybum marianum (L.) Gaertn.	Milk thistle	Peeled basal leaves	Stewed, seldom raw in salads
Taraxacum obovatum (Willd.) DC.	Dandelion	Basal leaves	Raw (salads)
CANNABACEAE			
Humulus lupulus L.	Hop	Young shoots with little leaves	Stewed (omelettes)
CARYOPHYLLACEAE			
Silene vulgaris (Moench.) Garcke	Bladder campion	Young stems with leaves	Stewed, seldom raw in salads
CHENOPODIACEAE			
Beta maritima L.	Sea beet	Basal leaves	Stewed
CUCURBITACEAE			
Bryonia dioica Jacq.	Red bryony	Young shoots with little leaves	Stewed
DIOSCOREACEAE			
Tamus communis L.	Black bryony	Young shoots with little leaves	Stewed; seldom raw
LILIACEAE			
Allium ampeloprasum L.	Wild leek	Bulbs and bottom of leaves	Stewed (omelettes); sometimes raw or as a condiment
PAPAVERACEAE			
Papaver rhoeas L.	Poppy	Young stems with leaves	Stewed; seldom raw in salads
POLYGONACEAE			
Rumex papillaris Boiss. et Reut.	Sorrel	Basal leaves	Raw (snack, salads)
Rumex pulcher L.	Fiddle Dock	Basal leaves	Stewed

subjected to reduction with L-cysteine to transform the DHAA in AA and analyse the total vitamin C content.

The analytical equipment used was a liquid chromatographer (Micron Analítica, Madrid, Spain) equipped with an isocratic pump (model PU-II), an AS-1555 automatic injector (Jasco, Japan), a Sphereclone ODS (2) 250 × 4.60, 5 µm Phenomenex column, a UV–visible detector (Thermo Separation Specta Series UV100); and software Biocrom 2000 3.0. The mobile phase was 1.8 mM H_2SO_4 (pH = 2.6). For AA analysis a flow-rate of 0.9 ml/min and UV detection at 245 nm was used, while conditions for organic acids were 215 nm UV detection and 0.4 ml/min flow rate. Linear calibration curves were obtained for quantification purposes from solutions with known amounts of all the identified compounds (AA, oxalic, malic, citric and fumaric acids), in in *m*-phosphoric acid to prevent the oxidation of AA. Figure 2 shows the chromatographic profiles obtained.

The quality assurance of analytical methods applied consisted on a validation procedure, including chromatographic characteristic of the peaks obtained. All these parameters were acceptable, according to Hsu and Chien (1994), with a capacity factor between 1 and 1.5, resolution higher than 6, and a tailing factor between 0.9 and 1.3. Calibration curves were linear in a concentration range of 10–200 mg/100 g, with r^2 >0.998 and coefficients of variation of response factors below 4%. Mean recovery percentages were evaluated, ranging between 90.1 and 94.6%, being in the interval accepted by the Food and Drug Administration for substances around 100 ppm (90–107%). Repeatability and reproducibility assays gave rise to coefficients of variation below the limit of 5.3%

Fig. 1 Some of the wild vegetables analysed. *Silybum marianum*: **a** plant at gathering time; **b** and **c** pealing the midribs and preparing the sample. *Anchusa azurea*: **d** and **e** collecting tender basal leaves. *Tamus communis*: **f** stem with leaves and female flowers; **g** bundle of young shoots (gathered before flowering). *Asparagus acutifolius*: **h** asparagus and plant

maximum recommended for substances around 100 ppm (AOAC 1993). Methods were sensitive enough to determine the amounts of organic acids in the samples, showing the following limits of detection (expressed as mg/100 g of fresh plant material): 0.414 mg/100 g for oxalic acid; 0.171 mg/100 g for malic acid; 0.039 mg/100 g for citric acid; 0.031 mg/100 g for fumaric acid, and 0.0762 mg/100 g for ascorbic acid.

All the procedures of extraction and analysis were performed in triplicate and data were subjected to statistical analysis using the Statgraphics plus for Windows (5.1 version).

Results and discussion

In general, leafy vegetables with high moisture content are tender and succulent, while those with low moisture have a harder texture. Dry matter content of wild vegetables analysed ranged between 6.40 and 29.69% (Table 2), being under 16% for most of the species, and only higher than 18% in the case of *Scolymus hispanicus* and *Allium ampeloprasum*. On the contrary, *Silybum marianum* stands out with very low dry matter values (6.80%), and so very high moisture content, due to the fleshy midribs (peeled basal leaves) of this vegetable. These results are in agreement with those reported by other authors related to dry matter content (in a range of 7–29%) for different species of wild leafy vegetables (Guil et al. 1997; Escudero and de Arellano 2003).

As shown in Table 2, pH was a parameter with low variability in all the species analysed, being most of them in a range between 4.5 and 6, with significant differences ($P < 0.05$) for *Humulus lupulus* (mean 6.58) and the two species of *Rumex* which showed the lowest pH accompanied by a high titratable acidity, especially *R. papillaris* (pH of 2.97 and 15.94 meq NaOH/100 g), a kind of endemic sorrel from Central Spain. The last figure clearly explains the very acid taste of this species, also indicated by its Spanish name, *acedera*. *Rumex pulcher* presented a mean pH of 4.67 and a mean titratable acidity of 3.85 meq

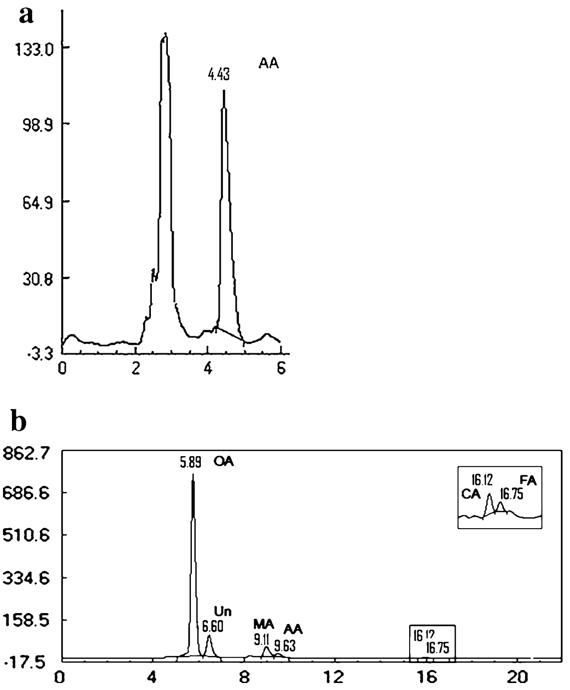


Fig. 2 **a** HPLC profile of vitamin C (ascorbic acid) in *Rumex papillaris*; **b** HPLC profile of organic acids in *Rumex papillaris*. **a** Chromatographic conditions: Sphereclone ODS(2), (250 × 4.60 mm), 5 µm column; mobile phase 1.8 mM H_2SO_4 (pH = 2.6); $\lambda_{detection} = 245$ nm; flow rate = 0.9 ml/min; AA: Ascorbic Acid. **b** Chromatographic conditions: Sphereclone ODS(2), (250 × 4.60 mm), 5 µm column; mobile phase 1.8 mM H_2SO_4 (pH = 2.6); $\lambda_{detection} = 245$ nm; flow rate = 0.9 mL/min; *OA* Oxalic Acid, *Un* Unknown, *MA* Malic Acid, *CA* Citric Acid, *FA* Fumaric Acid

NaOH/100 g. For the rest of the analysed species, mean titratable acidity was between 0.89 (*Scolymus hispanicus*) and 2.06 meq NaOH/100 g (*H. lupulus*). Acidity may be influenced by environmental factors as differences were obtained for some of the species in the two localities (see Table 2).

Table 3 shows the results of organic acids and Vitamin C found in the thirty samples analyzed, corresponding to the fifteen wild vegetables collected in the two different sites. As shown in Fig. 3, the mean values for total organic acids contents in the analysed species were very different, ranging between 23 mg/100 g (*Chondrilla juncea*) and 607 mg/100 g (*Beta maritima*). Together with the latter, *Papaver rhoeas* and *S. marianum* reached the highest mean values for total organic acids and, as will be treated afterwards, also the highest content of oxalic acid. As can be seen in Table 3, some variability between the two localities was found for each species, probably due to variations in the metabolic status of the plants.

Some authors have previously stated that the acidity of plants is mainly due to citric and malic acids (Oliveira et al. 2008). Souci et al. (2008), reported citric and malic as principal acids in some vegetables, as *Brassica oleracea* and *Asparagus officinale* (350 and 60 mg/100 g of citric acid and 200 and 95 mg/100 g of malic acid, respectively). In our study, four different organic acids were identified and quantified: oxalic, malic, citric and fumaric acid, as it has been previously described by other authors for tomato fruits (Fernández-Ruiz et al. 2004). Each species showed a qualitative and quantitative different organic acid profile, in agreement with previous works.

As can be seen in Fig. 3, almost all the species have a predominant organic acid, contributing to more than 75% of the total organic acids content. Citric acid represented 90% of total organic acids in *Tamus communis* (211 mg/100 g). Malic acid (ranging between 1.7 and 303.12 mg/100 g) was more than 60% of total organic acids content in *H. lupulus*, *Taraxacum obovatum* and *Cichorium intybus. Humulus lupulus* stands out ($P < 0.05$) from the rest of the species for its very high malic acid values (close to 300 mg/100 g). Fumaric acid was always the minor one, with no significant differences between species (always below 10 mg/100 g) with the exception of *S. marianum* and *Bryonia dioica*.

Oxalic acid is a major compound in many leafy vegetables and, in general, a very wide variability in this organic acid content has been found in the literature (Guil et al. 1996; Guil et al. 1997). In this study it appeared as predominant in nine of the species analysed, being 97% of the organic acids in *S. marianum* (563 mg/100 g), which presented the highest content of this compound, followed by *B. maritima* and *P. rhoeas* (526 and 459 mg/100 g, respectively). Guil et al. (1997) also reported more than 500 mg/100 g in *B. maritima* samples gathered in areas of South-eastern Spain, with very different environmental conditions (a drier and hotter climate) than those of the collecting areas of this study. Other species with oxalic acid as the main one were *R. pulcher* and *R. papillaris,* in agreement with the results presented by other authors for *Rumex induratus* (Ferreres et al. 2006). The oxalic acid values

Table 2 Physicochemical characteristics of wild vegetables analysed

Species	Location	Dry matter (g/100 g)	pH	Titratable acidity (meq NaOH/100 g)
Foeniculum vulgare	A	9.93 ± 0.15	5.71 ± 0.01	1.55 ± 0.38
	B	13.61 ± 0.38	5.74 ± 0.02	1.62 ± 1.89
Chondrilla juncea	A	10.22 ± 0.06	4.58 ± 0.04	1.55 ± 0.56
	B	26.26 ± 1.70	5.98 ± 0.06	0.63 ± 0.05
Cichorium intybus	A	11.96 ± 0.02	6.21 ± 0.02	0.30 ± 0.56
	B	12.61 ± 0.01	4.62 ± 0.01	1.67 ± 0.30
Scolymus hispanicus	A	19.67 ± 1.24	4.52 ± 0.02	1.21 ± 0.96
	B	29.69 ± 0.42	6.06 ± 0.04	0.58 ± 0.36
Silybum marianum	A	6.40 ± 0.14	5.38 ± 0.13	1.16 ± 0.17
	B	7.76 ± 0.65	4.65 ± 0.11	1.01 ± 0.20
Taraxacum obovatum	A	13.29 ± 0.22	5.93 ± 0.02	1.64 ± 0.41
	B	15.05 ± 0.30	5.58 ± 0.05	0.63 ± 0.42
Humulus lupulus	A	12.11 ± 0.13	6.34 ± 0.01	2.63 ± 0.35
	B	14.82 ± 0.19	6.82 ± 0.03	1.51 ± 0.66
Silene vulgaris	A	13.21 ± 0.20	5.67 ± 0.06	2.86 ± 0.27
	B	11.92 ± 0.19	4.93 ± 0.02	0.36 ± 0.11
Beta maritima	A	10.87 ± 0.44	6.21 ± 0.03	0.64 ± 0.27
	B	14.96 ± 0.13	5.95 ± 0.06	2.26 ± 1.16
Bryonia dioica	A	10.55 ± 0.04	4.93 ± 0.04	2.39 ± 1.26
	B	10.17 ± 0.11	5.33 ± 0.35	0.38 ± 0.38
Tamus communis	A	13.59 ± 0.45	5.90 ± 0.02	1.62 ± 0.69
	B	16.68 ± 0.05	5.82 ± 0.05	1.56 ± 0.60
Allium ampeloprasum	A	18.49 ± 0.01	6.09 ± 0.05	1.00 ± 1.12
	B	23.97 ± 0.14	5.43 ± 0.04	1.32 ± 0.21
Papaver rhoeas	A	10.94 ± 0.03	6.22 ± 0.03	1.61 ± 1.78
	B	13.10 ± 1.71	4.82 ± 0.55	2.39 ± 2.01
Rumex papillaris	A	11.75 ± 0.05	2.90 ± 0.02	16.80 ± 13.21
	B	9.73 ± 0.16	3.03 ± 0.03	15.07 ± 10.35
Rumex pulcher	A	10.70 ± 0.01	4.56 ± 0.07	4.34 ± 2.95
	B	11.26 ± 0.31	4.78 ± 0.02	3.36 ± 2.41

Mean ± standard deviation, n = 3

Two populations (A and B) of each species were analysed

reported by Souci et al. (Souci et al. 2008) for cultivated species of *Taraxacum* (24 mg/100 g) and *Foeniculum vulgare* (5 mg/100 g) were, respectively higher and lower than those found in our samples.

Oxalic acid has a quite low toxicity, with 5 g as the minimal lethal dose for an adult (Guil et al. 1997). However, the presence of oxalates in plants can interfere with the absorption of calcium and contribute to the formation of oxalate kidney calculus. For that reason, the ingestion of high levels of this organic acid is not desirable. Some authors (Guil et al. 1996) recommended an oxalic acid/Ca relation not higher of 2.5 in the foods to avoid this negative effect.

From all the wild Spanish vegetables analysed in this study, a clear division ($P < 0.05$) can be established between low-oxalic acid content species, such as *Allium ampelloprasum, T. communis, T. obovatum, C. intybus, Chondrilla juncea* and *H. lupulus* (with less than 68 mg/100 g as a mean value of oxalic acid), and oxalic-rich species (over 100 mg/100 g). From this point of view, the

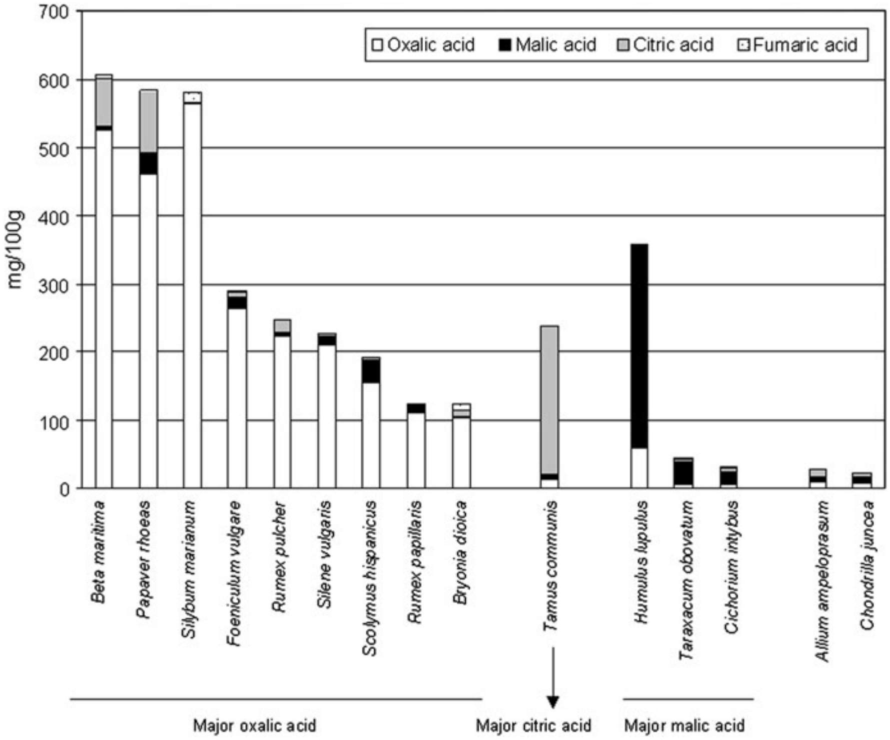
Table 3 Vitamin C and other organic acids found in wild vegetables analysed (mg/100 g)

Species	Batch	Oxalic	Malic	Citric	Fumaric	AA	DHAA	Total vitamin C
Foeniculum vulgare	A	123.82 ± 8.75	14.56 ± 1.67	7.64 ± 1.17	1.08 ± 0.12	10.68 ± 0.19	16.14 ± 3.05	26.82 ± 3.13
	B	402.83 ± 21.87	19.69 ± 0.53	5.66 ± 1.57	2.45 ± 0.05	16.94 ± 0.36	37.00 ± 2.65	54.14 ± 2.79
Chondrilla juncea	A	6.71 ± 0.80	8.14 ± 2.03	0.83 ± 0.267	0.42 ± 0.04	3.55 ± 0.20	18.45 ± 0.00	22.11 ± 0.00
	B	8.36 ± 1.46	12.43 ± 1.62	8.56 ± 0.507	0.51 ± 0.08	0.57 ± 0.01	10.56 ± 0.72	11.14 ± 0.73
Cichorium intybus	A	8.68 ± 0.05	11.14 ± 0.94	5.79 ± 0.703	0.64 ± 0.10	7.00 ± 0.28	15.16 ± 0.48	22.15 ± 0.47
	B	3.00 ± 0.71	25.87 ± 2.24	6.95 ± 0.018	1.27 ± 0.11	1.45 ± 0.53	18.83 ± 1.74	20.29 ± 1.31
Scolymus hispanicus	A	192.45 ± 26.38	35.22 ± 2.18	3.25 ± 0.007	0.91 ± 0.02	0.41 ± 0.71	1.14 ± 1.97	1.55 ± 2.68
	B	118.54 ± 7.18	26.99 ± 1.41	3.16 ± 0.000	0.90 ± 0.16	0.98 ± 0.09	3.81 ± 0.67	4.83 ± 0.61
Silybum marianum	A	662.03 ± 35.69	ND	ND	2.96 ± 0.18	1.04 ± 0.19	1.90 ± 0.29	2.94 ± 0.17
	B	464.50 ± 6.50	1.69 ± 0.03	1.49 ± 0.233	26.29 ± 0.52	ND	1.87 ± 0.14	1.87 ± 0.14
Taraxacum obovatum	A	3.30 ± 0.98	26.14 ± 3.01	5.12 ± 0.448	1.00 ± 0.11	1.84 ± 0.50	10.70 ± 1.28	12.53 ± 1.00
	B	7.84 ± 0.85	41.75 ± 13.65	2.81 ± 1.200	1.17 ± 0.21	1.46 ± 0.40	17.65 ± 0.92	19.53 ± 1.19
Humulus lupulus	A	50.80 ± 6.91	303.12 ± 84.96	ND	ND	14.45 ± 1.70	27.58 ± 0.21	42.03 ± 1.69
	B	67.78 ± 1.62	295.59 ± 0.00	ND	ND	11.12 ± 1.08	28.63 ± 0.62	39.32 ± 0.80
Silene vulgaris	A	201.79 ± 15.98	11.08 ± 0.15	1.94 ± 0.391	1.57 ± 0.12	27.66 ± 1.78	ND	27.66 ± 1.78
	B	218.73 ± 17.56	16.96 ± 0.91	4.63 ± 0.360	0.70 ± 0.01	16.37 ± 0.32	9.80 ± 2.76	26.32 ± 3.05
Beta maritima	A	543.02 ± 8.68	3.18 ± 0.43	12.42 ± 1.05	4.18 ± 0.64	10.37 ± 0.23	9.89 ± 1.45	20.26 ± 1.26
	B	509.32 ± 31.99	6.41 ± 0.62	130.87 ± 15.23	5.20 ± 0.46	10.02 ± 0.75	8.47 ± 5.33	18.49 ± 4.57
Bryonia dioica	A	123.85 ± 6.72	2.87 ± 0.26	3.36 ± 0.32	3.04 ± 0.05	20.27 ± 0.87	3.86 ± 2.72	24.13 ± 2.43
	B	83.08 ± 11.52	ND	19.43 ± 1.54	11.93 ± 2.87	15.16 ± 0.25	13.03 ± 0.00	28.19 ± 0.00
Tamus communis	A	11.97 ± 1.42	11.50 ± 1.22	229.27 ± 21.69	ND	52.37 ± 0.99	27.30 ± 1.10	79.44 ± 0.18
	B	12.35 ± 2.64	6.34 ± 0.43	207.24 ± 0.20	ND	48.73 ± 2.30	22.08 ± 3.11	70.81 ± 5.41
Allium ampeloprasum	A	11,13 ± 0.48	7.57 ± 0.83	11.77 ± 1.21	1.29 ± 0.15	4.23 ± 0.23	3.83 ± 0.21	8.06 ± 0.41
	B	6.32 ± 0.65	10.40 ± 1.14	9.44 ± 1.33	0.42 ± 0.06	3.49 ± 0.26	1.15 ± 0.16	4.77 ± 0.01
Papaver rhoeas	A	490.00 ± 27.05	24.78 ± 2.92	13.17 ± 1.78	1.69 ± 0.18	12.52 ± 0.52	5.64 ± 2.27	18.15 ± 1.88
	B	428.65 ± 63.63	43.79 ± 2.08	162.54 ± 12.52	4.00 ± 0.39	14.49 ± 0.77	16.15 ± 0.96	30.65 ± 1.24
Rumex papillaris	A	142.27 ± 11.81	11.39 ± 1.12	4.73 ± 0.13	ND	22.49 ± 3.30	2.85 ± 2.41	25.35 ± 0.89
	B	80.49 ± 17.09	9.87 ± 0.75	ND	ND	7.81 ± 0.50	14.42 ± 0.79	22.22 ± 1.26
Rumex pulcher	A	327.72 ± 42.50	3.21 ± 0.40	11.98 ± 0.91	ND	24.16 ± 0.72	4.27 ± 3.33	28.69 ± 2.54
	B	122.60 ± 29.64	5.10 ± 0.44	23.97 ± 1.43	0.24 ± 0.02	16.73 ± 1.92	12.98 ± 1.89	29.71 ± 3.75

Mean ± standard deviation, n = 3

AA ascorbic acid, *DHAA* dehydroascorbic acid, *ND* non detected

Fig. 3 Average organic acids content of the Spanish wild vegetables analysed (with the exception of AA and DHAA)



aforementioned species with low values of oxalic acid should be preferred in the diet, to avoid the possibility of Ca-complexing effect of this acid and the subsequent interference with calcium absorption, as well as its crystallization as urinary calculi.

Regarding vitamin C content (the sum of AA + DHAA), a range of 20–40 mg of vitamin C/100 g has been reported for cultivated leafy vegetables, with some exceptions as water-cress or purslane, which can reach more than 70 mg/100 g (Souci et al. 2008). In our study, a wide variability on vitamin C content was obtained among the analysed species, ranging from 1.5 to 79.4 mg/100 g. The species of the Asteraceae family had a significantly lower content of ascorbic acid ($P < 0.05$).

Table 3 and Fig. 4 show that the distribution between AA and DHAA is also highly variable. AA was the major form in all the analysed species, except for *F. vulgare, H. lupulus* and the species of the *Asteraceae* family. From this family, *S. marianum* and *Scolymus hispanicus* showed the lowest total vitamin C content (below 5 mg/100 g), together with *Allium ampeloprasum* (with a mean value of 6.35 mg/100 g), which however had more than half of the vitamin C content in the reduced form of AA. The low values of vitamin C in plants of the Asteraceae are different from those found by Escudero and de Arellano (2003) or Souci et al. (2008), who reported higher values (53 mg/100 g) in some Argentinean samples of *Taraxacum officinale*.

As can be seen in Fig. 4, among the species with AA as the major form of vitamin C, *T. communis* stands out with the highest mean value of vitamin C (75.12 mg/100 g). The reduced form (AA) reach a mean value of 50.91 mg/100 g (67% of total vitamin C), higher than many vitamin C sources commonly used in the human diet. The total vitamin C content in 100 g of the samples of *T. communis* analysed in this work is in the range of the recommended daily intake (RDI) for adults (75 mg/day for women and 90 mg/day for men), according to the Food and Nutrition Board (2000). *Silene vulgaris, B. dioica* and *R. pulcher* also standed out by their high AA proportion (66–81% of total vitamin C).

On the other hand, *F. vulgare* and *H. lupulus*, having less than 40% of vitamin C in AA form, presented still very high values of mean total vitamin C (40.48 and 40.68 mg/100 g, respectively). These values represented about 50% of RDI for adults (Food and Nutrition Board, Institute of Medicine 2000).

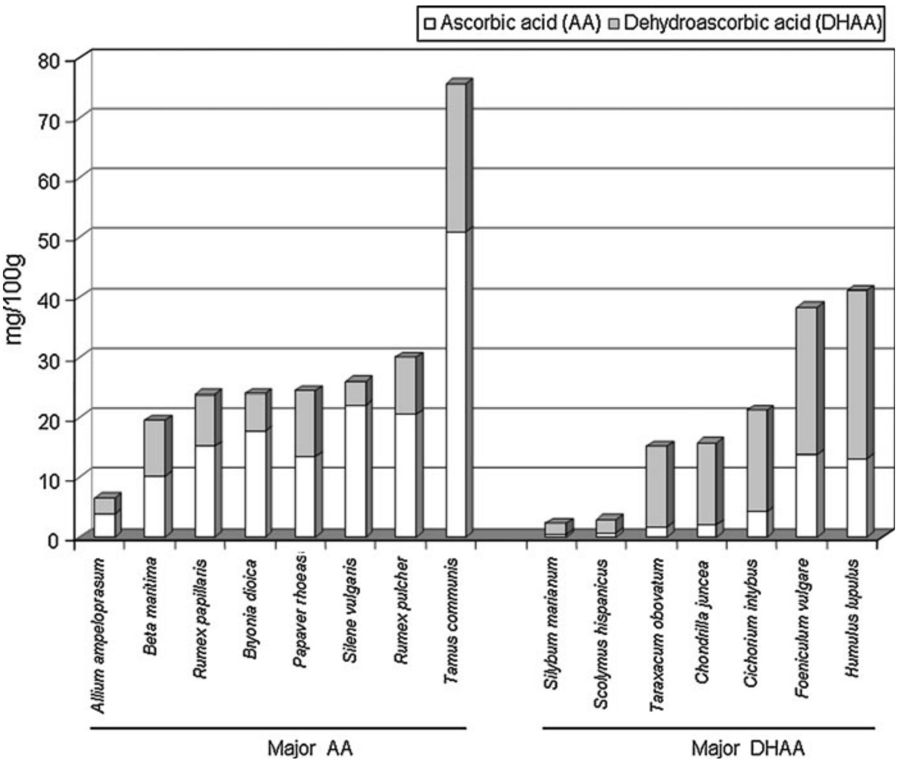


Fig. 4 Vitamin C content in the wild vegetables analysed (average values)

When comparing the average values of some wild species with their cultivated relatives (Mataix et al. 1998; Souci et al. 2008) there are many wild species that reach double values than cultivated ones, such as thistle (*S. marianum*); or 4 times greater, as in the case of chicory (*C. intybus*). The values of vitamin C, as well as the ratio AA/DHAA, obtained for *F. vulgare* were in agreement with the values obtained for cultivated varieties of the same species (8.7–34 mg/100 g) by Koudela and Petříková (2008), who also found high variability in vitamin C contents. On the contrary, Souci et al. (2008) reported higher values of vitamin C in *F. vulgare* (93 mg/100 g), *Taraxacum officinale* (68 mg/100 g) and *Allium porrum* (24 mg/100 g) than those found in this study.

It should be taken into account that two of the species with highest vitamin C content are toxic plants. Some fresh parts of *T. communis* (black bryony) contains saponins (diosgenin) and some parts of *B. dioica* (white bryony) may contain triterpene glycosides, as well as some ribosome inactivating proteins responsible of their toxicity (Hylands and Kosugi 1982; Siegall et al. 1994; Hadad Chi and Moradi 2005). However, these two species have been traditionally consumed in the Mediterranean area since ancient times (Tardío et al. 2006). In both cases the edible part are the young shoots, which are the least toxic parts of these plants, since toxic principles are more abundant in other plant organs, such as fruits and subterranean parts. Moreover, they are always consumed after a cooking process, which is supposed to destroy the toxic principles (Biglino and Nano 1965; Couplan 1990; Lin et al. 2006).

To study the potential relationship between vitamin C (AA + DHAA), the rest of the organic acids and the physicochemical characteristics analysed, a correlation analysis was carried out (Table 4). Total vitamin C, AA and DHAA were highly correlated, meaning that although the distribution of the two forms of vitamin C may be quite different among species, a high level of AA is usually accompanied by DHAA and therefore total vitamin C. The correlation between vitamin C and AA was the strongest one, suggesting that AA is usually the major form of this vitamin in the analysed species. A significant positive correlation between total vitamin C and AA and citric acid has been also found in these species (Table 4).

Table 4 Correlations coefficients and *P*-values (in brackets) between the variables analyzed

	D. M.	T. A.	pH	CA	MA	OA	FA	AA	DHAA
T. A.	−0.22								
	(0.25)								
pH	0.30	−0.74*							
	(0.11)	(0.00)							
CA	0.05	−0.08	0.13						
	(0.80)	(0.69)	(0.50)						
MA	0.26	−0.12	0.26	−0.04					
	(0.17)	(0.54)	(0.16)	(0.83)					
OA	−0.35	−0.09	0.03	0.09	−0.24				
	(0.06)	(0.65)	(0.87)	(0.63)	(0.20)				
FA	−0.25	−0.11	−0.13	−0.07	−0.22	0.31			
	(0.19)	(0.57)	(0.49)	(0.71)	(0.24)	(0.09)			
AA	−0.17	0.15	0.02	0.67*	−0.20	−0.06	−0.20		
	(0.38)	(0.43)	(0.91)	(0.00)	(0.30)	(0.76)	(0.30)		
DHAA	−0.12	−0.11	0.28	0.30	0.39*	−0.25	−0.21	0.31	
	(0.52)	(0.58)	(0.13)	(0.11)	(0.04)	(0.19)	(0.26)	(0.09)	
T. VIT C	−0.18	0.05	0.16	0.63*	0.06	−0.17	−0.25	0.87*	0.74*
	(0.34)	(0.79)	(0.39)	(0.00)	(0.74)	(0.37)	(0.18)	(0.00)	(0.00)

DM Dry matter; *T. A* Titratable acidity, *CA* Citric acid, *MA* malic acid, *OA* oxalic acid, *FA* fumaric acid, *AA* ascorbic acid, *DHAA* dehydroascorbic acid, *T. VIT C* total vitamin C

* Significant correlations ($P < 0.05$)

Conclusions

According to the results of this study, each species showed a specific organic acids fingerprint, with citric acid as characteristic of *T. communis*, and malic and oxalic acids as the major ones in the different species analyzed. *Tamus communis, F. vulgare* and *H. lupulus* may have higher vitamin C levels than many vitamin C sources commonly used in the human diet. *Rumex pulcher, S. vulgaris, B. dioica* and specially *T. communis,* also stand out for their AA content. These species could be therefore selected for their high nutritional interest. *Silene vulgaris* and *F. vulgare* have a special relevance since they can be eaten raw, with a better preservation of their AA and total vitamin C content. On the other hand, although young shoots of *T. communis, B. dioica* and *H. lupulus*, must be consumed cooked, they have very high levels of AA and vitamin C. Moreover, *H. lupulus* has less than 67 mg/100 g of oxalic acid, and so it could be selected as a species with high vitamin C and low oxalic acid, for their inclusion in the diet as a vegetable, or as a source of new functional ingredients in developing new foodstuffs.

This study represents a preliminary survey to obtain nutritional data for some Spanish wild vegetables, not previously studied or scarcely reported. More research is needed to investigate other aspects of the chemical composition (nutritional and pharmacological value) and agronomic potential of these neglected and underutilized plant genetic resources.

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