Journal of Applied Botany and Food Quality 88, 109 - 114 (2015), DOI:10.5073/JABFQ.2015.088.015

[1]Dipartimento di ScienzeFarmaceutiche, Via Romana, Università degli Studi di Perugia, Perugia, Italy
[2]Dipartimento di Ingegneria Civileed Ambientale, Università degli Studi di Perugia, Perugia, Italy
[3]Dipartimento di Scienze Agrarie Alimentaried Ambientali, Perugia, Italy

Bioactive compounds and antioxidant characterization of three edible wild plants traditionally consumed in the Umbria Region (Central Italy): *Bunias erucago* L. (corn rocket), *Lactuca perennis* L. (mountain lettuce) and *Papaver rhoeas* L. (poppy)

Angela Maurizi[1], Alfredo De Michele[1], Aldo Ranfa[2], Anna Ricci[2], Valentina Roscini[3], Roberto Coli[1], Mara Bodesmo[2*], Giovanni Burini[1]

(Received November 19, 2014)

Summary

The leaves of three edible wild plants, *Bunias erucago* L. (Corn rocket), *Lactuca perennis* L. (Mountain lettuce) and *Papaver rhoaes* L. (Poppy) were analysed for their proximate composition, some nutraceutical components and total antioxidant capacity. The protein levels ranged from 2.7 to 4.1 g/100 g of the edible portion. The range of dietary fibre content was 3.8 to 6.4 g/100 g of the edible portion. The amount of ash, carbohydrate and lipid ranged from 1.7 to 1.9, 3.3 to 4.4 and 0.22 to 0.45 g/100 g of the edible portion, respectively. Lipids consisted mainly of polyunsaturated fatty acids with the highest value for *Bunias erucago* L. (71.8% of total fatty acids) and *Lactuca perennis* L. (70.0%). Potassium (374.0-521.0 mg/100 g) and calcium (204.8-331.8 mg/100 g) were the most representative macro-elements in the species studied. The values of vitamin E, β-carotene and total vitamin C were included in the range from 0.91 to 2.61 mg/100 g, from 1,957 to 2,631 μg/100 g and from 19.2 to 31.0 mg/100 g, respectively. Our results showed that the total antioxidant capacitywhich ranges from 27.2 to 63.7 μmol TE/g, according to the Oxygen Radical Absorbance Capacity (ORAC) method, is highly justifiable due to the high content of phenolic compounds (159-246 mg GAE/100 g).

Introduction

The use of edible wild plants had a fundamental role, linked to survival, in many past civilisations. Although in recent decades their consumption has been limited, today interest in them has been rehabilitated due to their healthy properties. Plants constitute an important source of active natural products that differ widely in terms of structure and biological properties. A diet rich in vegetables produces beneficial effects (Kaur and Kapoor, 2001) because some of their constituents counteract the action of reactive oxygen species (ROS) implicated in numerous degenerative pathologies such as cardiovascular diseases, diabetes, cancer and neurodegenerative disorders (Halliwell and Cross, 1994). Edible wild plants are a good source of nutrients and nutraceutical compounds, such as vitamin C, vitamin E (α-tocopherol), β-carotene (pro-vitamin A) and polyphenols. Vitamin C, E and β-carotene have various biological functions and antioxidant activities (Sies and Stahl, 1995; Paiva and Russel, 1999). In phytochemicals, polyphenols are among the richest group and possess various biological effects including antioxidant activity (Kahkonen et al., 1999). Epidemiological studies suggest that diets rich in polyphenols are associated with potential neuroprotective benefits (Vauzour et al., 2008), reduced incidence risk of cardiovascular diseases (CHDs) (Who) and specific types of cancer (Franceschi et al., 1997). The lipids of edible wild plants are mainly constituted by unsaturated fatty acids such as omega-3 fatty acids which are essential for normal growth and development and may play a fundamental role in many diseases (Simopoulos, 2004). Dietary fibre is an important fraction and shows protective effects on different diseases (Kendall et al., 2010), including colorectal cancer, cardiovascular disease (Theuwissen and Mensink, 2008), diabetes, obesity, and diverticular disease.

Wild food plants provide a qualitative contribution to the traditional Mediterranean diet (Pieroni et al., 2005). Potential health effects of these herbs are shown in several studies supported by the European Commission (Heinrich et al., 2006; Hadjichambis et al., 2008).

In recent years, many researchers have shown an increased interest in wild plants for several reasons: the renewed interest in local traditional foods (see for example Guarrera et al., 2005; Ghirardini et al., 2007; Cornara et al., 2009; Guarrera et al., 2009), the potential of these foods as nutraceuticals, and in the prevention degenerative diseases.

Consequently, the aim of the present study was to evaluate the chemical composition, some bioactive compounds and the antioxidant activity in three selected edible wild plants, collected in winter in their natural habitat (in Umbria, Central Italy, on the slopes of Mount Subasio, in the Perugia area).

Materials and methods

Collection and preparation of samples

Three different samples of edible wild plants, *Bunias erucago* L. (Corn rocket), *Lactuca perennis* L. (Mountain lettuce) and *Papaver rhoaes* L. (Poppy), were collected in the wild nature in February 2012, analysed with a Stereomicroscope SX45, and classified according to the Checklist of Italian Vascular Flora (Conti et al., 2007). All the *exsiccata,* of the aforementioned species are preserved in the Erbario PERU of the Perugia University. These three species were chosen because they are among the most representative and best known in traditional folk recipes and are used both raw, in salads, and cooked, in soups, or as boiled greens. Each sample was a bunch of several leaves collected from different plants to ensure a representative sample of each species. Moreover, the leaves were preventively washed with distilled water to remove all foreign materials such as soil and sand. Depending on the component to be determined, the analysis was carried out on the fresh or lyophilised leaves. A weighed portion (100 g) was lyophilised for 24 h (Minifast 2000 Edwards Global Headquarter, Crawley, UK) and the dry weight was determined.

Chemical composition

The moisture content of the three edible wild plants was determined by drying the leaves in an oven at 100 °C until a constant weight ac-

* Corresponding author

cording to AOAC method (AOAC, 1990) was obtained. Crude protein content was calculated using the conversion factor 6.25 (Nx6.25) from the nitrogen content determined by the Kjeldahl method (AOAC, 1990). Total lipid was evaluated using chloroform/methanol extraction based on the Bligh and Dyer procedure as described in the AOAC method (AOAC, 1990). Ash content was determined according to the AOAC method (AOAC, 1990) by incineration in a muffle furnace at 500 °C for 6 h. Total dietary fibre was determined by the enzymatic-gravimetric method according to the AOAC method (AOAC, 1995). Carbohydrates were calculated by difference. According to the AOAC method (AOAC, 2006) iron, calcium and magnesium determination was conducted by AA-6800 Model flame (air-acetylene) atomic absorption spectrophotometer (AAS) (Shimadzu, Kyoto, Japan) on the ashes which were obtained from 4 g of each lyophilised sample dissolved in 10 mL of 6 N of HCl. The solution was then transferred into a 50 mL volumetric flask and the volume was adjusted with distilled water. Calcium, magnesium and iron certified stock standard solutions, 1000 ppm in HNO_3 (CARLO ERBA, Milan, Italy) were used. For calcium analysis, lanthanum chloride (5%) was added to each sample to avoid interference with the phosphate. Standard working solutions were prepared according to the user manual in order to obtain the calibration curve. Sodium and potassium determination was conducted using a PFP7 Flame Photometer (Bibby Scientific, Jenway, Techne Inc., UK) on the same solution. Sodium and potassium certified stock industrial standard solutions were purchased from Jenway (Jenway, Essex, UK). Standard working solutions were prepared according to the user manual in order to obtain the calibration curve. Analysis accuracy was confirmed for all the metals studied using certified standard reference material (NIST-SRM-1573rd, tomato leaves). Phosphorus content was determined colorimetrically on 0.2 g of each lyophilised sample by reading the absorbance at 650 nm using ammonium molybdate, hydroquinone and sodium sulphide solutions according to the AOAC method (AOAC, 1990). Polyphenols were determined by the Folin-Ciocalteu method with measurement at 760 nm with a spectrophotometer (Varian UV/Vis 50 Cary Bio model, Palo Alto, CA, USA). The results were expressed as mg of gallic acid equivalent (GAE) per 100 g fresh weight (SINGLETON and ROSSI, 1965). The assay was carried out on 250 mg of each lyophilised sample.

Fatty acid composition

Fatty acids were determined by high-resolution gaschromatography (HRGC) with a flame ionisation detector (FID) and a capillary column. Fatty acid methyl esters (FAMEs) were prepared from the lipid extract by a transesterification process catalysed with sulphuric acid (FREEDMAN et al., 1986). The fatty acid profile was analysed with a Fisons Instruments model HRGC MEGA 2 Series (Fisons Instruments, Altrincham, UK) equipped with a split-splitless injector with temperatures of the injector and detector at 300 °C. Separation was achieved on a 30 m × 0.25 mm i.d. fused silica capillary column coated with a 0.25 μm film of DB WAX 122-7032 columns (Agilent Technologies, Delaware, USA). Helium was used as carrier gas at 1 mL/min. The column temperature was programmed from 130 °C (for a 1 min hold) to increase to 180 °C at a rate of 5 °C/min (for a 5 min hold), then to increase to 230 °C at a rate of 4 °C/min (for a 40 min hold) and finally to 250 °C at a rate of 2 °C/min (for a 5 min hold). The standard and the sample were injected in the splitless mode, and the injected volume was 0.5 μL. The results are expressed in the relative percentage of each fatty acid, calculated by internal normalisation of the chromatographic peak area. Fatty acid identification was made by comparing the relative retention times of FAME peaks from the samples with standards. A NuCheck Prep (NuCheck Prep, Inc., MN, USA) mixture of FAMEs standard was used.

Simultaneous determination by normal phase-high performance liquid chromatograph (NP-HPLC) of tocopherols and β-carotene

Tocopherols and β-carotene were determined by a modified NP-HPLC (REDI, 1999) operating with a Spectra Physics SP8800 Model ternary pump (Mountain View, CA, USA), and a Rheodyne 7125 (COTATI, CA, USA) sample-injection valve with a 10 L injection loop. Two detectors were joined in series in order to determine, with a single run, the tocopherols (α-, β-, γ- and δ-tocopherol) and β-carotene. Tocopherols were detected by a JASCO FP-920 Model, Tokyo, Japan fluorometric detector (FLD) equipped with a light source of 150 W xenon lamp, $\lambda_{Ex.} = 290$ nm and $\lambda_{Em.} = 330$ nm while β-carotene was detected by a UV/Vis detector (SHIMADZU SPD-10 A VP Model, Kyoto, Japan) set at 450 nm. A personal computer with appropriate software (Varian Star Chromatograph ver. 6.00 Walnut Creek, CA, USA) for data acquisition and processing was used. Isocratic separation was achieved on a Merck Hibar, LiChrosorb Si 60, 250 mm x 4 mm i.d., 5 m particle size column (Merck, Darmstadt, Germany) using a mixture of *n*-hexane and 2-propanol (98:2, v/v) as eluent at a flow-rate of 1 mL/min. The standard stock solutions containing 500 μg/mL of each tocopherol was prepared by dissolving 50 mg of each α-, β-, γ- and δ-tocopherol (Calbiochem a brand of EMD Biosciences, Inc., an affiliate of Merck KGaA, Darmstadt, Germany) in a 100 mL volumetric flask with absolute ethanol (J.T. BAKER, Mallinckrodt Baker, Milan, Italy). Solutions containing 0.25, 0.50, 1.00, 2.00, 4.00 and 8.00 μg/mL of each tocopherol, used for the calibration curve, were prepared by appropriate dilution with *n*-hexane (J.T. Baker, Mallinckrodt Baker, Milan, Italy). A standard stock solution, containing 250 μg/mLof β-carotene (Calbiochem a brand of EMD Biosciences, Inc., an affiliate of Merck KGaA, Darmstadt, Germany), was prepared by dissolving 25.8 mg of β-carotene in 100 mL in a volumetric flask with *n*-hexane. Solutions containing 0.10, 0.20, 0.40, 0.80, 1.60 and 3.20 μg/mL of β-carotene, used for the calibration curve, were prepared by appropriately diluting the stock solution with *n*-hexane. This solution was kept at -20 °C and made freshly every week. The extraction of tocopherols and β-carotene from the samples was carried out by the addition of 25 mL of *n*-hexane to 0.5 g of fresh leaves of each sample placed in a Falcon 2070 50 mL polypropylene graduate conical tube (Falcon, Boston Dickinson Labware, NJ, USA). The mixture was homogenised with an Ultra Turrax (IKA TI 25, Milan, Italy) to obtain a fine suspension and then was centrifuged (Eppendorf 5810 R Model, Milan, Italy) at 4000 rpm for 5 min. Two mL of the supernatant solution were transferred into a screw-cap tube (10 mL) and the *n*-hexane was evaporated under a mild stream of nitrogen at 30 °C; dry residue was dissolved in 0.5 mL of mobile phase. The resulting solution was analysed by NP-HPLC. The data, collected by Workstation software (Varian Star Chromatograph software version 6.00, Walnut Creek, CA, USA), were processed to quantify the tocopherols and β-carotene with the external standardisation method.

Total L-ascorbic acid determination

Total L-ascorbic acid (AA) determination was carried out according to the BURINI method (2007), based on radical oxidation of L-ascorbic acid (AA), to obtain dehydro-L-ascorbic acid (DHAA) by means of a peroxyl radical generated *in situ* by thermal decomposition of an azo-compound, 2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH). The DHAA is condensed with benzene-1,2-diamine (*o*-phenylendiamine, OPDA) to form its highly fluorescent quinoxaline derivative, 3-(1,2-dihydroxyethyl)furo[3,4-b]quinoxaline-1-one (DFQ) which was then separated by HPLC with fluorometric detection (FLD). The sample was prepared by homogenising 1 g of fresh leaves in a Falcon 2070 50 mL polypropylene graduate conical tube (Boston Dickinson Labware, NJ, USA) with 35 mL of 1% (w/v)

metaphosphoric acid (MPA). The resultant mixture was transferred into a 50 mL volumetric flask and brought to volume with 1% (w/v) MPA. An aliquot of 3 mL of each extract was filtered (Sartorius 0.45-m membrane filter) and 1 mL of filtrate was analysed.

Total antioxidant capacity determination by Hydrophilic-Oxygen Radical Absorbance Capacity (H-ORAC). The total antioxidant capacity by ORAC assay was based on the radiative fluorescence decay of the fluorescein (fluorescent probe) produced by reactive oxygen species (ROO•) formed *in situ* by thermal decomposition of an azo-compound in the presence of an antioxidant as a reference standard (Trolox) and a sample. The antioxidant compounds of the sample, acting as free radical scavengers, produce a stable signal of fluorescence that decreases more or less rapidly according to their concentration and reactivity. The data points of fluorescence intensity (FI) were summarised over time by the evaluation software. The total value of the sum FI of each sample, subtracting the total value of the sum of blank, was integrated on the calibration curve to obtain the ORAC value expressed as micromoles of Trolox Equivalents per gram (μmol of TE/g). The assay was conducted with FLUOstar Optima fluorescent microplate reader (BMG LABTH GmbH, Germany), provided with a pump, set to $\lambda_{Ex.}$ =485 nm and $\lambda_{Em.}$ =520 nm and interfaced with a computer provided with a MARS Data Analysis software ver. 2.00 (BMG LABTH GmbH, Germany) for data acquisition and processing. Costar 96 well black opaque plates (Corning Costar Corporation, Cambridge, MA, USA) were used. Two hundred μL of 60 nM fluorescein (Sigma-Adrich, Steinheim, Germany) working solution was added to all experimental designated in the wells. In addition, blank wells received 20 μL of 75 mM phosphate buffer (pH 7.2), while standard wells received 20 μL of each 10, 20, 40 and 80 mM Trolox (a vitamin E analogue hydrosoluble, 6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxyl acid) (Sigma Aldrich, Steinheim, Germany) standard solution in phosphate buffer and each sample well received 20 μL of solution as indicated below. Four wells for the blank, four wells for each standard solution of Trolox and four wells for the three samples for a total of 32 wells were used. The plate was then allowed to equilibrate by incubating for 30 min at 37 °C and then the fluorescence in each well was read by plate reader (2 cycles). In the next cycle, the pump was programmed to inject 60 L of 160 mM of AAPH freshly prepared solution into the respective wells to start the reactions. The data were collected every 2.3 min by monitoring the fluorescence. Each sample extract was obtained adding 20 mL of a CH_3COCH_3: H_2O: CH_3COOH (70:29.5:0.5 v/v/v) mixture (Prior et al., 2003) to 0.5 g of each fresh leaf sample in a 50 mL polypropylene graduate conical tube (Falcon 2070 Blue Max, Boston Dickinson Labware, NJ, USA). The mixture was subjected to homogenisation (IKA TI 25 Ultra Turrex, Milan, Italy) to obtain a fine suspension. After centrifuging at 4000 rpm for 5 min (Eppendorf centrifuge 5810 R Model, Eppendorf, Milan, Italy) the supernatant was diluted 1:50 with phosphate buffer and the resulting solution was used for ORAC evaluation.

Statistical analysis

Data are presented as mean ± standard deviation (SD). Each analytical determination was replicated three times except for the H-ORAC assay that was carried out in quadruple. The statistical analysis of the data was performed using the Statistical Analysis System (SAS release 8.1, SAS Institute Inc. Cary, North Carolina, USA). A paired Student *t*-test was used to compare the mean values. A value of $P<0.05$ was considered significant.

Results and discussion

The species analysed was characterised by moderate amounts of proteins, low amounts of lipids, considerable amounts of minerals and acceptable fibre content. For the species analysed statistically significant differences among protein, lipid and dietary fibre content were observed ($P<0.05$). Tab. 2 shows that lipids were represented mainly by such polyunsaturated fatty acids and particulary by α-linolenic acid (C18:3n-3) and linoleic acid (C18:2n-6). *P. rhoeas* L. had a high oleic acid content (C18:1n-9) and a higher amount of linoleic acid compared to *B. erucago* and *L. perennis*. Linolenic acid was higher in *B. erucago* compared to *P. rhoeas* and *L. perennis*. The surprising presence of C20:5n-3 (EPA) in *L. perennis* and in *P. rhoeas* was probably due to the extraction method (Aoac, 1990), that allows the extraction of membrane polar lipids, important components of chloroplast.

Tab. 3 shows signifcant differences in the content of various minerals. It can be observed that potassium and calcium were the main minerals, followed by phosphorus, magnesium and sodium. Statistically significant differences were observed in Ca content for *L. perennis* and in Mg content for *B. erucago* unlike the other species. Iron content was important, especially in *B. erucago*, similar if not higher than that of meat, even though of lesser bioavailability. Only a few studies have been published regarding some compositional characteristics of *P. rhoeas* (Zeghichi et al., 2003; Akrout et al., 2010) while analytical data for *B. erucago* and *L. perennis* are not available in the literature. Substantial differences between our data and those reported by Zeghichi et al. (2003) and Akrout et al. (2010) were found in all mineral concentrations for *P. rhoeas*. This is probably due to different factors such as the harvest period, parts studied (leaves, stems, flowers) and to different pedo-climatic conditions where the plants grow.

Dietary fibre amount was satisfactory because it contributes to the satisfaction of recommended daily intake (Sinu, 1996). (Tab. 3).

Our results obtained for phenolic compounds (see Tab. 4) suggest that these important bioactive components are contained in great amounts, from 159 to 246 mg GAE/100 g of the edible portion.

Statistically significant differences between *L. perennis* and the other species were observed. Tab. 4 shows the results of α-tocopherol and β-carotene found in the three samples analysed. The maximum values of α-tocopherol and β-carotene were found in *L. perennis*.

Tab. 1: Chemical composition of raw edible wild plants (g/100g edible weight).

Plant name	Moisture	Protein	Lipid	Carbohydrate	Ash	Dietary fibre
Bunias erucago L. (Corn rocket)	86.4±0.51	4.1±0.10	0.22±0.01	3.3±0.54	1.7±0.15[a]	4.3±0.23[a]
Lactuca perennis L. (Mountain lettuce)	84.2±0.44	2.7±0.12	0.45±0.02	4.4±0.39	1.8±0.11[a]	6.4±0.30
Papaver rhoeas L. (Poppy)	87.2±0.64	3.5±0.20	0.32±0.02	3.3±0.28	1.9±0.09[a]	3.8±0.27[a]

[a] means followed by the same superscript are not significantly different at 0.05 level.

Tab. 2: Most representative fatty acids of the raw edible wild plants (%of total fatty acids).

Abbreviation	Common name	*Bunias erucago* L. (Corn rocket)	*Lactuca perennis* L. (Mountain lettuce)	*Papaver rhoeas* L. (Poppy)
$C_{12:0}$	Lauric	tr.	0.49±0.11	tr.
$C_{14:0}$	Myristic	0.73±0.12	2.12±0.15	0.40±0.09
$C_{15:0}$	-	0.28±0.04	0.40±0.08	tr.
$C_{16:0}$	Palmitic	19.51±1.15	18.15±1.02	16.30±1.11
$C_{16:1n-7}$	Palmitoleic	0.33±0.06	0.33±0.05	0.14±0.03
$C_{17:0}$	Margaric	0.24±0.05	tr.	tr.
$C_{18:0}$	Stearic	1.04±0.09	1.92±0.12	2.81±0.20
$C_{18:1n-9}$	Oleic	2.41±0.20	2.77±0.22	12.12±0.62
$C_{18:1n-7}$	-	2.82±0.25	0.45±0.07	0.69±0.06
$C_{18:2n-6}$	Linoleic	12.35±0.76	24.45±0.93	28.06±0.94
$C_{18:3n3}$	a-Linoleic	59.42±1.48	45.57±1.70	37.49±1.56
$C_{18:3n-4}$	Stearidonic	tr.	0.27±0.04	0.20±0.04
$C_{20:0}$	Arachidic	0.32±0.05	1.20±0.11	0.54±0.08.
$C_{20:5n-3}$	EPA	tr.	1.14±0.10	0.56±0.08

tr. = trace

Tab. 3: Mineral content of raw edible wild plants (g/100g edible weight).

Plant name	K	Na	Ca	Mg	Fe	P(*)
Bunias erucago L. (Corn rocket)	374.0±30.39[a]	10.9±0.77	228.5±16.79[a]	20.4±1.99	5.9±0.69	69.6±6.56[a]
Lactuca perennis L. (Mountain lettuce)	440.8±38.28[a,b]	47.4±3.20	331.8±21.06	31.6±2.52[a]	3.3±0.20	47.4±4.51
Papaver rhoeas L. (Poppy)	521.0±33.07[b]	28.2±2.72	204.8±20.15[a]	30.7±2.84[a]	4.1±0.41	75.3±4.74[a]

(*) Total phosphorus.
[a] means followed by the same superscript are not significantly different at 0.05 level.

Tab. 4: α-tocopherol, β-carotene, total ascorbic acid, total phenol content and antioxidant capacity of raw edible wild plants. In parentheses the repeatability relative to standard deviation (RSD_r) is shown.

Component	*Bunias erucago* L. (Corn rocket)	*Lactuca perennis* L. (Mountain lettuce)	*Papaver rhoeas* L. (Poppy)
α-tocopherol, mg/100 g	0.91±0.05 (5.5)	2.61±0.14 (5.4)	1.72±0.10 (5.8)
β-carotene, mg/100 g	1,957±78 (4.0)	2,631±121 (4.6)	2,176±105 (4.8)
Ascorbic acid, mg/100 g	31.0±0.99(3.2)	19.2±0.79 (4.1)[a]	21.3±1.10 (5.2)[a]
Total phenols, mg GAE/100 g	159±7 (4.4)	246±15 (6.1)	188±11 (5.9)
ORAC, mmol TE/g	27.24±1.55 (5.7)	63.70±2.81 (4.4)	43.89±2.32 (5.3)

[a] means followed by the same superscript are not significantly different at 0.05 level.

A few authors have determined α-tocopherol in edible wild plants (Simopoulos, 2004) by analysing the edible wild plants of Crete. They found a lower value than that of our data, probably due to many different environmental factors and to the parts of the plant studied. α-Tocopherol values determined in our group were similar to values found in broccoli (1.44 mg/100 g) and spinach (1.96 mg/100 g), which were the highest values of other raw vegetables studied (Chun et al., 2006). Fig. 1 shows the HPLC of standard tocopherol solutions (Channel A) and β-carotene (Channel B). A good separation of α-tocopherol and the other tocopherols was obtained. Fig. 2 refers, as an example, to *L. perennis* analysed in the same conditions. As can be seen from Fig. 2, and similarly in the other two species examined, just α-tocopherol was found. Tab. 5 shows the parameters relating to the linear regression, the limit of detection (LOD) and the limit of quantification (LOQ) for tocopherols and for β-carotene.
The limits of detection (LOD) were evaluated, according to the Federal Register (1997), on the standard deviations of the response and the slope using the ratio $3.3\ \sigma/S$, where σ = the standard deviation of the response and *S*=the slope of the calibration curves of α-, β-, δ- and γ- tocopherol and β-carotene, respectively. The *σ*

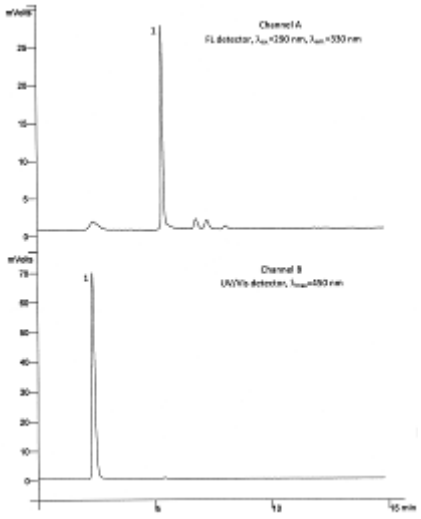

Fig. 1: Chromatogram of standard tocopherols (2.00 μg mL^{-1} of each) and β-carotene (0.80 μg mL^{-1}). Channel A: 1=α-tocopherol, 2=β-tocopherol, 3=γ-tocopherol, 4=δ-tocopherol. Channel B: 1=β-carotene.

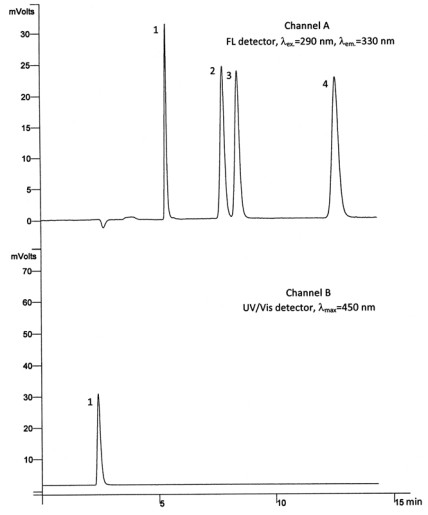

Fig. 2: Typical chromatogram of *L. perennis* Channel A: 1=α-tocopherol (2.16 μg mL^{-1}). Channel B: 1=β-carotene (2.20 μg mL^{-1}).

values were estimated on the calibration curves using the standard deviations of the *y*-intercepts of the regression lines. The limit of quantification (LOQ) was calculated by using the ratio *10* σ/*S*. Precision, considered as repeatability, was determined by analysing three replicates of all the samples analysed. The method used showed good repeatability of the relative standard deviations (RSD_r), which in each instance was ≤5.8% for both α-tocopherol and β-carotene. Furthermore, accuracy was high considering the good recoveries obtained by spiking known amounts of tocopherols and β-carotene to the samples. Recoveries were included for α-, β-, δ- and γ-tocopherol in the ranges 99.0-102.8%, 98.5-103.6%, 97.1-100.2%, 99.2-102.3% and in the range 96.8-97.9% for β-carotene, respectively. The results of total L-ascorbic acid found in the three samples analysed are also reported in Tab. 4; the contents are in the range 19.2-31.0 mg/100g of fresh leaves. Statistically significant differences between *B. erucago* and the other species were observed. The content of total L-ascorbic acid was similar to the values that are found for raw celery (32 mg/100 g), raw chard (24 mg/100 g), raw cultivated asparagus (18 mg/100 g) and raw chicory (17 mg/100 g) (INRAN, 2000). In particular, the value obtained for *P. rhoeas* was comparable with that reported by SANCHEZ MATA et al., 2012. The results of ORAC, reported in Tab. 4, show higher values (the maximum for *L. perennis*) compared to the values of other vegetables relevant to nutrition (NINFALI et al., 2005). Although ORAC assay allowed us to obtain lipophilic (L-ORAC) and hydrophilic (H-ORAC) antioxidant fractions (PRIOR et al., 2003), our study was focussed on the hydrophilic fraction, which was the most important quantitative portion; indeed, in vegetables the L-ORAC fraction is very low compared to H-ORAC (WU et al., 2004) justifiable by the low content of lipids in the samples examined. The standard solutions of Trolox show linearity from 10 to 80 M. The equation of the calibration curve, obtained from the sum of the fluorescence intensity *versus* the Trolox concentrations, was y=13,392+2,524x with a good correlation coefficient (R^2) of 0.9928. The repeatability relative standard deviation (RSD_r) of samples was good; in each instance $RSD_r$was ≤5.7%. The limitation of the total antioxidant capacity determination of foods lies in the fact that it is assessed *in vitro* far from reproducing the biological conditions of the human organism. It does not take into consideration metabolic reactions, which largely influence bioavailability of antioxidant components. To confirm the beneficial effects of the bioactive components contained in edible wild plants, it would be useful to observe *in vivo* plasma antioxidant capacity. A research of this kind, carried out in our laboratory on lyophilised and reconstituted red wine, produced a significant increase in plasma antioxidant capacity due to its bioactive constituents (ALBERTI FIDANZA et al., 2003). Plasma antioxidant capacity has been suggested as a good *in vivo* marker of the antioxidant status (FERNANDEZ PATCHON et al., 2008).

Conclusion

In conclusion the leaves of the wild edible plants, *B. erucago*, *L. perennis* and *P. rhoaes*, possess important nutraceutical compounds which enhance their nutritional properties. Their use in the usual diet can help providing beneficial effects to human health due to the presence of dietary fibre, vitamin C, vitamin E, β-carotene, potassium, calcium and polyphenols and to their high total antioxidant capacity. Our results can also be used to expand and update the food composition database.

A new, interesting property of the wild species examined is represented by the presence of an adequate concentration of antioxidant components capable of contrasting the effect of free radicals. Thanks to their antioxidant properties, it would certainly be worthwhile to perform further studies of edible wild plants and to promote commercialisation campaigns, particularly in view of the growing demands by the food industry for natural antioxidants. Human health in general could greatly benefit from a reconsideration of these wild plants because they represent a naturally-occurring, easy to obtain source of powerful vegetable antioxidants.

Acknowledgements

We are grateful to Prof. Giovanni Gigliotti, University of Perugia, for his intellectual support and Gina Pero for her technical support.

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Address of the corresponding author:
Mara Bodesmo, Dipartimento di Ingegneria Civile ed Ambientale, Università degli Studi di Perugia, Borgo XX Giugno, 06121 Perugia, Italy
E-mail: bodesmo@hotmail.it