Food Chemistry 123 (2010) 416–422

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Levels of antioxidants and nutraceuticals in basil grown in hydroponics and soil

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ARTICLE INFO

Article history:
Received 30 September 2009
Received in revised form 4 March 2010
Accepted 26 April 2010

Keywords:
Ocimum basilicum
Ascorbate
Lipoic acid
Phenolic acids
Tocopherol
DPPH
Fremy's salt
$ABTS^{+\cdot}$

ABSTRACT

Owing to the traditional phytotherapeutic use of basil and its importance as a basic component of the Mediterranean diet, this research aimed to study the nutraceutical properties of basil (*Ocimum basilicum* cv. Genova) grown in hydroponics in comparison with that grown in soil. The antioxidant activities of aqueous and lipid extracts of basil leaves were evaluated both by spectrophotometric detection with the 2,2′-azinobis(3-ethylbenzothiazoline-6-sulphonic acid) radical cation ($ABTS^{+\cdot}$) and by electron paramagnetic resonance (EPR) detection with the stable radicals peroxylamine disulphonate (Fremy's salt, hydrophilic) and 1,1-diphenyl-2-picrylhydrazyl ($DPPH^{\cdot}$, lipophilic). From EPR decay kinetics analysis, it was possible to distinguish (in the lipid extract) a fast rate constant and a slow rate constant, likely attributable to two different kinds of lipophilic antioxidants. Hydroponic cultivation improved antioxidant activity of both aqueous and lipid extracts, increasing the contents of vitamin C, vitamin E, lipoic acid, total phenols and rosmarinic acid.

1. Introduction

Antioxidants have been widely used as food additives to avoid food degradation, and they play an important role in preventing many lifestyle-related diseases and ageing, being closely related to the formation of reactive oxygen species (ROS) and to lipid peroxidation (Noguchi & Niki, 1999). However, there are concerns about the use of synthetic antioxidants, such as butylated hydroxy anisole (BHA) and butylated hydroxy toluene (BHT) because of their instability and their possible activity as promoters of carcinogenesis (Barlow, 1990). Consequently, during recent years, there has been much interest in the antioxidant activity of naturally occurring substances (Sgherri, Pinzino, Izzo, & Navari-Izzo, 2007b).

Basil (*Ocimum basilicum* L.), a member of the Lamiaceae family, is an annual crop widespread in Asia, Africa, South America and the Mediterranean region. It is widely cultivated in many countries under natural and greenhouse conditions. Cultivation in greenhouses maximises the yield and allows a constant supply of the material throughout the year.

Basil is a very important medicinal plant and culinary spice, and is marketed fresh, dried or frozen. Traditionally, basil has been used as a medicinal plant in the treatment of headache, cough, diarrhoea and kidney malfunctions (Simon, Chadwick, & Craker, 1984), against insect bites, acne (Waltz, 1996), and it has long been used to flavour foods, as well as dental and oral products (Simon et al., 1984).

It is a common opinion that the origin of most illnesses is due to the generation of free radicals, natural products of aerobic metabolism, that increase following pathological events such as inflammation. Generally, antioxidants stop the progress of free radical chain reactions, helping to prevent the development of pathological events. The interest in vitamins C, E, lipoic acid and phenols is due to their antioxidant properties (Sgherri, Kadlecova, Pardossi, Navari-Izzo, & Izzo, 2008; Sgherri, Navari-Izzo, Pardossi, Soressi, & Izzo, 2007a), which strongly determine their biological functions in both plant and animal metabolism. In fact, they can interact enzymatically and non-enzymatically with damaging oxygen free radicals and their derivatives, thus protecting plants from oxidative stress and mammals from oxidative stress-related diseases, such as cancer, cardiovascular pathologies and ageing (La Vecchia, 1997). Antioxidative effects are a result of the capacity of antioxidants to inhibit the initiation of free radical processes, or to interrupt the chain reactions in the propagation of oxidation. In plant cells, two different types of antioxidants can be distinguished: hydrophilic types, such as vitamin C and lipophilic types, such as vitamin E and carotenoids. During recent years, the detection of plant extract antioxidant activity has been achieved by the use of electron paramagnetic resonance (EPR) (Gardner, McPhail, & Duthie, 1998).

Ocimum basilicum contains phenolic compounds (Loughrin & Kasperbauer, 2001; Tarchoune et al., 2009) that act as powerful

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doi:10.1016/j.foodchem.2010.04.058

antioxidants, free radical-scavengers, and metal chelators (Rice-Evans, Miller, & Paganga, 1996; Sgherri, Cosi, & Navari-Izzo, 2003; Tsai, Tsai, Yu, & Ho, 2007). The concentrations of vitamins and phenolics in basil leaves are influenced by many factors, including soil, irrigation and climatic conditions. Hydroponics gives guarantees reproducibility by uniformity of growth conditions (greenhouse conditions) and a better control of the different kinds of fertilisation (homogeneity of nutrient concentrations). The cultivation of plant material in hydroponics ("floating system"), which now has practical applications in Tuscany (Italy), is aimed to gain advantages from a production point of view and in terms of quality, as shown by some preliminary studies for *Echinacea angustifolia* DC (Sgherri, 2006, personal communication). This kind of cultivation, carried out under control conditions, also eliminates problems linked to the disinfection of soil and balance of nutrients encountered in natural soil due to the non-homogeneous distribution of nutrients.

Owing to the traditional phytotherapeutic use of basil and its importance as a basic component of the Mediterranean diet (typical example is the "Genoese pesto"), this research aims to study the nutraceutical properties of basil (*O. basilicum* cv. Genova) grown in hydroponics in comparison with that grown in soil. The antioxidant activity of aqueous and lipid extracts of basil leaves are evaluated, both by spectrophotometric detection with the 2,2′-azinobis(3-ethylbenzothiazoline-6-sulphonic acid) radical cation ($ABTS^{+\cdot}$) and by EPR detection with the stable radicals peroxylamine disulphonate (Fremy's salt, hydrophilic) and 1,1-diphenyl-2-picrylhydrazyl ($DPPH^{\cdot}$, lipophilic). In particular, special attention is focused on the study of vitamin C, vitamin E, lipoic acid and phenolic acid composition because of the remarkable contributions of these compounds to the antioxidant potential of vegetables.

2. Materials and methods

2.1. Chemicals

All reagents were of the highest purity and were purchased from Sigma–Aldrich (Milan, Italy). Water was of Milli Q grade. All solvents and water were accurately degassed before use in the analyses.

2.2. Plant materials

Plants (*O. basilicum* L. cv. Genova), grown in hydroponics and in soil, were collected during summer, 2007, in the farm "Azienda Agricola Le Campore", situated in Massarosa (LU, Italy), especially equipped for cultivation in "floating system". Glasshouse conditions were maintained with a minimum night temperature and a daytime ventilation temperature of 16 and 28 °C, respectively, and a relative humidity of 75%. A mean value of daily radiation of 9.2 MJ/m^2 with a maximum photon flux density of 500–700 $\mu mol/m^2$ s was supplied. Nutritive solution had a pH of 7.5 and an electric conductivity of 2.5 mS/cm. It was mainly composed of 15 mM NO_3^-, 2 mM $H_2PO_4^-$, 4 mM SO_4^-, 1–5 mM NH_4^+, 4–5 mM Ca^{2+}, 2 mM Mg^{2+}, 3 mM Na^+ and 10 mM K^+. Microelements, such as Fe, B, Cu, Zn and Mn, were added at the concentrations of 20.6, 46.3, 2.4, 2.3 and 7.3 μM, respectively, using, as their sources, $FeSO_4$, H_3BO_3, $CuSO_4$, $ZnSO_4$ and $MnCl_2$. Leaves of basil, grown in hydroponics (H) and in soil (S), were collected randomly 35 days after sowing (35H and 35S), at the typical growth stage for farm supplies in the Tuscany market. For cultivation in a "floating system", a growth stage of 20 days after sowing (20H) was also considered because the Genovese market requires more tender plants.

2.3. Sample extracts

Fresh leaves were pulverised with liquid nitrogen, using a mortar. The aqueous extract was obtained using Milli Q water, accurately degassed, containing 5 mM diethyldithiocarbamic acid and 5 mM Na_2EDTA. After centrifugation at 12,100*g* for 15 min, the pellet was discarded and the supernatant was used for determination of the total antioxidant activity of the aqueous phase and phenolic acid composition. Lipid extraction was performed in the dark and under a continuous flux of nitrogen, using chloroform/methanol (2:1, v/v). The extract was washed three times with KCl 0.88% (w/v) in order to eliminate salts. Chloroform phases were taken to dryness with a rotary evaporator and resuspended in chloroform/ethanol (1:5, v/v). Soon after resuspension, the lipid extract was used for determination of antioxidant activity of total lipid phase and tocopherol composition.

2.4. Antioxidant activity

2.4.1. EPR analysis

EPR spectra were recorded with a Varian E112 (X-band) spectrometer equipped with a Varian variable temperature accessory. The spectrometer was interfaced to an AST Premium 486/25 EISA computer by means of an acquisition board (Ambrosetti & Ricci, 1991) and a software package designed for EPR measurements (Pinzino & Forte, 1992). Computer-based simulations of EPR spectra were performed using the Winsim programme (Duling, 1994). Spectra were recorded at 25 °C in a 1 mm quartz sample tube sealed at one end inserted into the microwave cavity of the spectrometer. EPR parameters used were: microwave power, 10 mW; microwave frequency, 9.16 GHz; modulation amplitude, 1 Gauss; central magnetic field, 3265 Gauss. Fremy's salt and $DPPH^{\cdot}$ were employed to determine antioxidant potential in aqueous and lipid extracts, respectively. In the incubation mixture, the final concentration of Fremy's salt was 0.5 mM and that of $DPPH^{\cdot}$ 3.3 mM. In order to know the decay of the radical before measuring antioxidant activity, decay kinetics of Fremy's salt or $DPPH^{\cdot}$, with and without the extract, were recorded. The amplitude of the central line of Fremy and $DPPH^{\cdot}$ spectra was taken for registration of kinetics. Antioxidant potential was reported as number ($n°$) of $DPPH^{\cdot}$ or Fremy's radicals reduced by 1 g (dry weight, DW) of plant material.

2.4.2. Spectrophotometric analysis

Radical cation $ABTS^{+\cdot}$ was generated as described by Pellegrini, Re, Yang, and Rice-Evans (1999). The radical solution was diluted in ethanol or water for lipid or aqueous extracts, respectively, in order to obtain an absorbance at 734 nm of 0.70 ± 0.05. After addition of the extract, the decrease in absorbance was monitored and compared to that of the trolox standard. Antioxidant activity was expressed in terms of trolox equivalent antioxidant capacity (TEAC)/g DW of plant material.

2.5. Antioxidants

2.5.1. Ascorbic acid (vitamin C)

Basil leaves were immediately homogenised at 4 °C in ice-cold 6% trichloroacetic acid (w/v) using a cold mortar. After centrifugation at 12,000*g* for 30 min, total ascorbate + dehydroascorbate (DHA) and reduced ascorbate (AsA) were immediately determined in the supernatants according to Nakagawara and Sagisaka (1984). The assay was based on the reduction of Fe^{3+} to Fe^{2+} by AsA in an acid solution. Fe^{2+} reacts with bathophenanthroline and forms a red compound. After incubation at 30 °C for 90 min, its absorbance was read at 534 nm. Total ascorbate (AsA + DHA) is determined by reduction of DHA to AsA by dithiothreitol (DTT) and DHA levels were estimated on the basis of

the difference between total ascorbate and AsA values. Calibration curves for AsA and DHA in the range of 5–50 nmol were used.

2.5.2. Lipoic acid

Both lipoic acid (LA) and dihydrolipoic acid (DHLA) were extracted from basil leaves by acidic hydrolysis according to Vianey-Liaud, Kobrehel, Sauvaire, Wong, and Buchanan (1994). After hydrolysis, samples were extracted with chloroform, following the procedure of Sgherri, Quartacci, Izzo, and Navari-Izzo (2002). The resultant organic phases were collected, evaporated to dryness under vacuum and stored at 4 °C under nitrogen. LA and DHLA contents in the extracted solutions were determined by isocratic RP-HPLC, using a Shimadzu apparatus (model LC-20AD) with an electrochemical detector (Metrohom model 791) equipped with a glassy carbon electrode and LC Solution software (Shimadzu) for integration of peaks. Detection was performed at +1.1 V at 25 °C with a Nova Pak C-18 4 μm column (3.9 mm × 150 mm). Extracts were eluted at 25 °C at a flow rate of 1 ml/min, using 24% (v/v) acetonitrile, 3% (v/v) 2-propanol, and 72% (v/v) 0.05 M KH_2PO_4 as mobile phase (pH 2.5). Chromatographic peaks were identified by comparing both retention times and spectra with those of the standards. Co-chromatography of the standards with the samples was also used to identify peaks with close retention times. Mixtures of standards of LA and DHLA in the range of 4–100 ng in 20 μl of injection volume were injected to calculate the calibration curve.

2.5.3. Tocopherols (vitamin E)

Tocopherols were determined in lipid extracts prepared as in Section 2.3. The four tocopherol forms (α-, β-, γ- and δ-) were determined by isocratic RP-HPLC, using a Shimadzu apparatus (model LC-20AD) with an electrochemical detector (Metrohom model 791) equipped with a glassy carbon electrode and LC Solution software (Shimadzu) for peaks integration. Detection was performed according to Galatro, Simontacchi, and Puntarulo (2001) at +0.6 V at 25 °C with a Nova Pak C-18 4 μm column (3.9 mm × 150 mm). The extracts were eluted with 95% methanol containing 20 mM $LiClO_4$ at a flow rate of 1 ml/min. For identification of peaks, the retention times and maximum spectra of tocopherols were compared with those of standards, which were also used for quantification. Standard mixtures of α-, β-, γ- and δ-tocopherol in the range of 25–75 ng in 20 μl of injection volume were injected to calculate the calibration curve.

2.5.4. Phenolic acids

Before analysis, aqueous extracts were passed through a Sartorius (Goettingen, Germany) filter (Minisart 0.45 μm) to remove any suspended material.

Qualitative and quantitative analyses were performed by a RP-HPLC (Talcott & Howard, 1999). Twenty microlitres were injected into a Waters model 515 HPLC system fitted with a 3.9 mm × 150 mm Nova-Pak C18 column (Waters, Milford, MA, USA). Detection was at 280 nm, using a Waters 2487 dual λ UV–visible detector. Mobile phase A contained 98% water and 2% acetic acid, and mobile phase B contained 68% water, 30% acetonitrile and 2% acetic acid. A linear gradient of 0–30% mobile phase B was run for 30 min at 1 ml min^{-1}. The identity of the phenolic acids was confirmed by co-chromatography on HPLC with authentic standards, and quantification was performed using a standard curve in the range of 0.1–0.5 μg of standard mixtures containing gallic, protocatechuic, *p*-hydroxybenzoic, chlorogenic, vanillic, caffeic, siringic, *p*-coumaric, ferulic and rosmarinic acids. Chromatogram analysis was performed by the software Millennium 32 (Waters).

2.5.5. Total phenols

A measure of total phenols in aqueous extract was obtained by recording absorbance at 280 nm (A_{280}) before and after the addition of insoluble polyvinylpoly-pyrrolidone (PVP) to the extract (1:10 w/v) so as to absorb the phenolic compounds (Sgherri et al., 2003). Calculation was performed by subtracting the unspecific absorbance of the extract, after removal of phenols by centrifugation at 12,000*g* for 30 min, from that of the un-treated extract and referring to a calibration curve for total phenols prepared with gallic acid as standard.

2.6. Statistical analysis

The results are the means from three replicates of three independent experiments ($n = 9$). All data are reported as mean values ± SE. The significance of differences among mean values was determined by one-way ANOVA. Comparisons among means were performed using Duncan's multiple range test. Reported means in tables and figures accompanied by different letters are significantly different at $P \leqslant .05$. When necessary, an arc sin or angular transformation was applied before statistical analysis was performed.

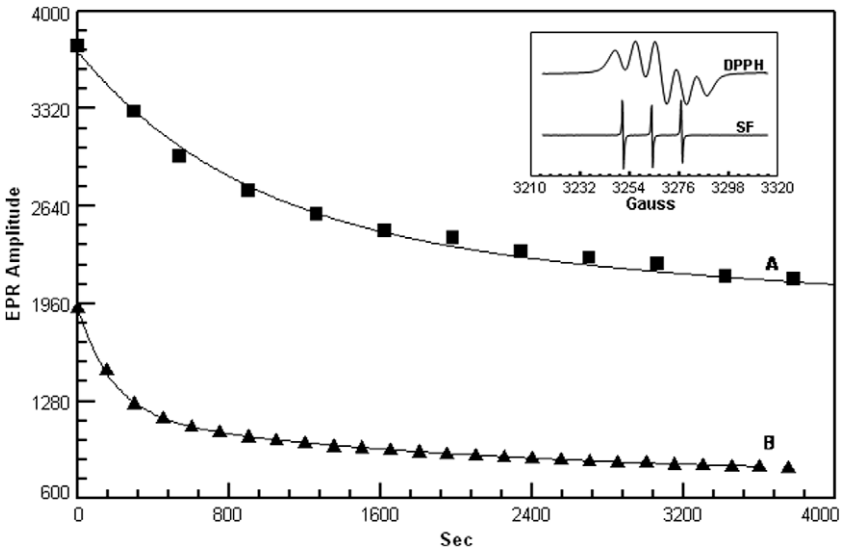

Fig. 1. EPR decay kinetics of 0.5 mM Fremy's salt (SF, A) and 3.3 mM 1,1-diphenyl-2-picrylhydrazyl (DPPH·, B) after the addition of 1.25 and 35 μl of aqueous and lipid extracts of basil leaves (20H), respectively. Inset: EPR spectra of SF and DPPH·.

3. Results

EPR spectra of DPPH· and Fremy's salt are shown in Fig. 1. They are characterised by 5 and 3 lines which are narrow enough, compared to the spectrum acquisition time, to be used for registration of kinetics with many points close. Examples of decay kinetics of Fremy's salt and DPPH radicals with aqueous and lipid extract, respectively, from basil leaves (20H) are shown in Fig. 1. They were both pseudo-first-order kinetics. Kinetics of Fremy's salt was characterised by a decay rate constant of $11.4 \times 10^{-5}\ s^{-1}$ for 20H whereas the decay kinetics of DPPH· in the presence of lipid extract was due to the contribution of two pseudo-first-order kinetics, characterised for 20H by a fast rate constant and a slow rate constant of 55.6×10^{-5} and $5.2 \times 10^{-5}\ s^{-1}$, respectively.

In Fig. 2, the antioxidant activity, detected by EPR, is expressed as the ability to reduce radicals by 1 g (DW) of basil leaves. Antioxidant potential of aqueous extract was 40.3% higher in plants grown in hydroponics for 20 days (20H) than in those grown for 35 days (35H). A significant decrease, by 14.0%, was also observed in the antioxidant activity of aqueous extract from plants grown in soil for 35 days (35S) in comparison with plants of the same age grown in hydroponics (35H, Fig. 2A). The same trend was shown by spectrophotometric measurements of $ABTS^{+}$ radical cation scavenged by aqueous extract (Fig. 3A). The two rate constants which characterise the decay kinetics of DPPH· are indicative of the presence of antioxidants in the lipid extract distinguishable for a fast or a slow action. For this reason, we can calculate (by EPR kinetics) a fast (Fig. 2B) or slow (Fig. 2C) antioxidant activity of the lipid extract. The fast and slow antioxidant activities were of the same magnitude, even if they showed different trends, depending on age and on the type of cultivation (Fig. 2B and C). Substantial decreases (about 60%) in the fast antioxidant activity of lipid extract were observed in 35H compared to 20H and in 35S compared to 35H (Fig. 2B), whereas the trend of slow antioxidant activity reflected that of antioxidant activity detected spectrophotometrically (Figs. 2C and 3B).

20H showed higher amounts of the reduced form of ascorbic acid (AsA) and of total ascorbate (AsA + DHA, Fig. 4A), as well as of total phenols (Fig. 4B). Total ascorbate and total phenols were the same in 35H and 35S whereas AsA was reduced by more than 50% when plants were grown in soil (35S) compared to hydroponics (20H and 35H). Phenolic acid composition was significantly affected by age and cultivation type, even if all samples contained (in large amounts) both gallic and *p*-hydroxybenzoic acids (Table 1). 20H also showed important amounts of syringic and rosmarinic acids whereas protocatechuic and ferulic acids reached the lowest levels. In 35H, all phenolic acids analysed were present at low levels compared to 20H with the exception of chlorogenic acid which doubled. Marked increases were observed with cultivation in soil (35S) compared to 35H, especially for gallic, *p*-hydroxybenzoic, vanillic, chlorogenic and caffeic acids, and with the exception of rosmarinic acid which decreased by 41.2% (Table 1).

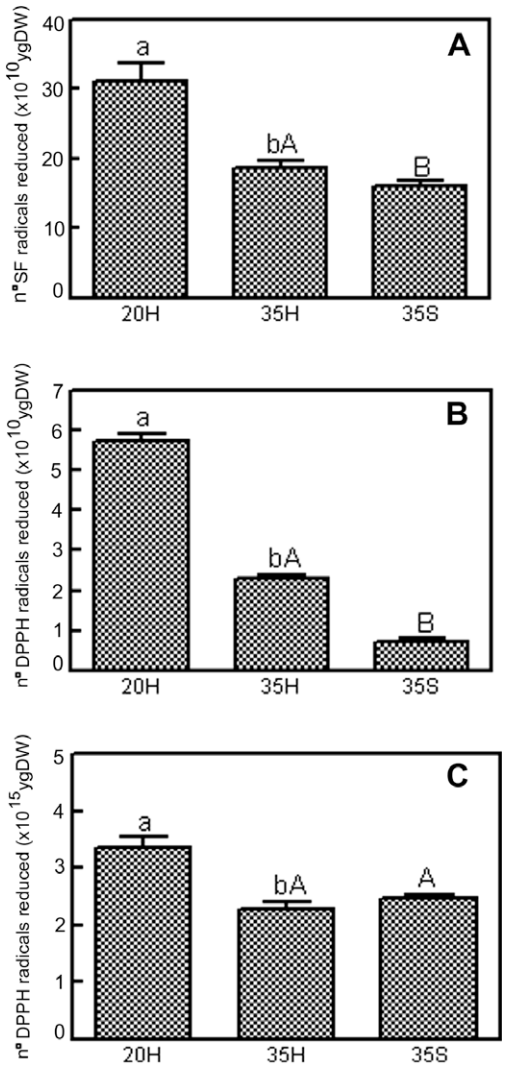

Fig. 2. Antioxidant activity of aqueous extract (A), and fast (B) and slow (C) antioxidant activities of lipid extract from basil leaves determined by EPR decay kinetics of Fremy's salt (SF) and 3.3 mM 1,1-diphenyl-2-picrylhydrazyl (DPPH·). 20H, basil grown in hydroponics for 20 days; 35H, basil grown in hydroponics for 35 days; 35S, basil grown in soil for 35 days. All data are reported as mean values ± SE. Reported means accompanied by different letters are significantly different at $P \leqslant .05$. Small letters and capital letters refer to differences between growth stages in hydroponics (20H and 35H) or differences between types of cultivation at the same age (35H and 35S).

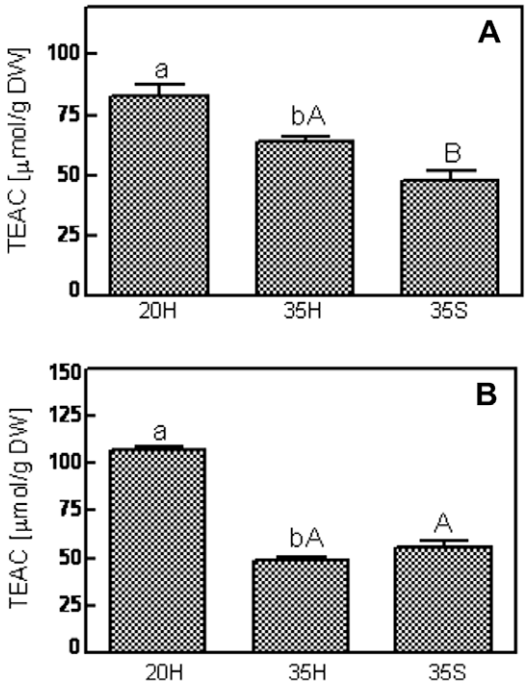

Fig. 3. Antioxidant activity of aqueous (A) and lipid (B) extract from basil leaves determined by decolourisation of 2,2′-azinobis(3-ethylbenzothiazoline-6-sulphonic acid) radical cation ($ABTS^{+\cdot}$). TEAC, trolox equivalent antioxidant capacity. The other details are as in Fig. 2.

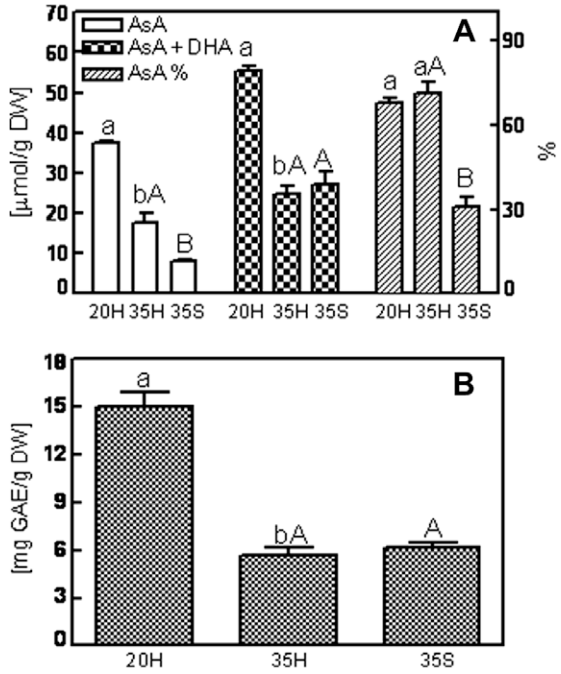

Fig. 4. Contents of total (AsA + DHA) and reduced (AsA) ascorbate (A, left axes), AsA% (A, right axes) and total phenols (B) in leaves of basil grown in hydroponics for 20 (20H) and 35 days (35H) and in soil for 35 days (35S). The other details are as in Fig. 2.

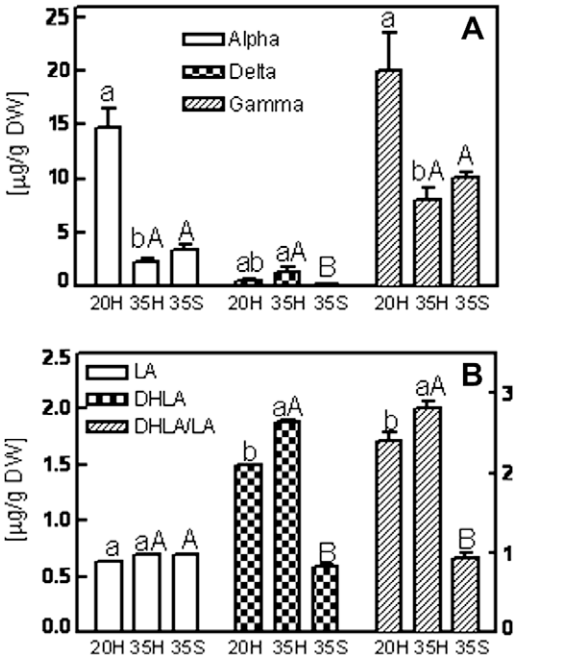

Fig. 5. Contents of tocopherols (A), lipoic (LA) and dehydrolipoic (DHLA) acid (B, left axes) and DHLA/LA ratio (B, right axes) in leaves of basil grown in hydroponics for 20 (20H) and 35 days (35H) and in soil for 35 days (35S). The other details are as in Fig. 2.

Major tocopherols determined in basil leaves were α- and γ-tocopherol; lower amounts were detected for δ-tocopherol whereas β-tocopherol was not detectable (Fig. 5A). 20H showed higher amounts of α- and γ-tocopherols, which decreased in 35H by 84.3% and 59.8%, respectively. No changes were observed in 35S compared to 35H with the exception of δ-tocopherol which showed a significant decrease in 35S (Fig. 5A).

Lipoic acid (LA) did not change in the different samples (Fig. 5B). However, its reduced form (DHLA) prevailed under hydroponic cultivation, reaching values 2.3- and 2.7-fold higher than LA in 20H and 35H, respectively. Under soil cultivation (35S), DHLA showed a value 68% lower than under hydroponic cultivation (35H) with a DHLA/LA ratio near to one (Fig. 5B).

4. Discussion

Horticultural production is increasingly oriented toward high quality products, in particular for the contents of phytonutrients (vitamins, antioxidants, minerals). Antioxidants, including vitamin C, vitamin E, phenols and lipoic acid, are responsible for the level of antioxidant capacity of a leaf extract. Thus, enhanced amounts of the biologically active reduced form of antioxidants can be indicative of an increased antioxidant capacity, i.e. the healthy properties, of basil leaves aqueous and lipid extracts (Figs. 1–3).

Table 1
Contents of phenolic acids (μg/g DW) in leaves from basil (*Ocimum basilicum* cv. Genova) cultivated in hydroponics for 20 (H20) and 35 days (H35) and in soil for 35 days (S35). Means in rows followed by different letters are significantly different at $P \leqslant .05$.

	20H	35H	35S
Gallic acid	208 ± 8.72 a	183 ± 5.35 bB	234 ± 7.93 A
Protocatechuic acid	15.8 ± 2.70 a	8.94 ± 1.93 bB	19.8 ± 3.54 A
p-Hydroxybenzoic acid	334 ± 7.85 a	269 ± 6.89 bB	334 ± 8.95 A
Vanillic acid	80.7 ± 4.87 a	56.8 ± 3.10 bB	106 ± 3.97 A
Chlorogenic acid	67.4 ± 2.78 b	137 ± 5.32 aB	522 ± 6.35 A
Caffeic acid	52.9 ± 7.31 ab	36.5 ± 6.98 bB	270 ± 6.83 A
Syringic acid	100 ± 3.67 a	10.4 ± 1.56 bA	9.32 ± 1.12 A
p-Coumaric acid	46.5 ± 3.56	tr.	tr.
Ferulic acid	29.1 ± 1.97	tr.	64.6 ± 4.32
Rosmarinic acid	150 ± 5.78 a	76.7 ± 3.76 bA	45.1 ± 2.48 B

Despite the spectrophotometric technique being less sensitive than EPR (Gardner et al., 1998; Sgherri et al., 2007b), antioxidant activity of aqueous and lipid extracts from basil leaves showed the same trend when detected by either EPR or spectrophotometry (Figs. 2 and 3). However, as previously observed on sage extracts (Sgherri et al., 2007b), the ratios between antioxidant activities of aqueous and lipid extracts from the same sample were different when determined by EPR or spectrophotometry (Figs. 2 and 3). This could be due to the presence of catechins which are known to scavenge the ABTS radical cation in the aqueous phase (Salah et al., 1995). Another reason could be the changes in reduction potentials of Fremy's radical, $DPPH^{\cdot}$ or $ABTS^{+\cdot}$ in different solvents used for determination of antioxidant activity in aqueous and lipid extract. In fact, the reduction potentials of the various hydroxyl groups on the polyphenols may change in different solvent systems, depending on their state of protonation or deprotonation. Electrostatic repulsion between the Fremy's radical anion and deprotonated moieties on the phenolics may also result in the different antioxidant activities of different systems under investigation (Gardner et al., 1998). The reduction potential of antioxidants can be altered by solvent conditions that change polarities and, thus, influence the pK_a values and molecular orbital energies (Gardner et al., 1998). Moreover, at pH below 7 (in the present experiment, pH was 6.45 in aqueous extract), the thiol group does not react with the ABTS radical and the antioxidant capacity of these compounds is lost (Arnao, Cano, Hernández-Ruiz, García-Cánovas, & Acosta, 1996). Differences in the literature have also been reported for TEAC values of basil leaves compared to those reported in the present paper (Fig. 3). Indeed, Nguyen and Niemeyer (2008) have found basil TEAC levels 3-fold higher than those reported here (Fig. 3). These discrepancies may be due to various extraneous reasons: agricultural and geographical conditions, different cvs, as well as growth conditions.

The use of an EPR spectrometer, equipped with a data acquisition system (Ambrosetti & Ricci, 1991) and a software package

especially designed (Pinzino & Forte, 1992) for analysis and simulation of spectra, also allowed us to monitor radical decay, due to the extract, over time. From analysis of the kinetics, it was possible to distinguish (in the lipid extract) a fast rate constant and a slow rate constant for the decay of DPPH·, likely attributable to the presence of two different kinds of lipophilic antioxidants, whose action is likely distinguishable by hydrogen atom transfer (HAT) or single electron transfer (SET, Prior, Wu, & Schaich, 2005). The nature of these antioxidants with different kinetic behaviours is not known and their identification is under investigation in our laboratory. The fact that tocopherol represents the most important lipophilic antioxidant and that the trend in the contents of its isomers reflects that of the fast antioxidant activity, might indicate tocopherol as one of the molecules responsible for this kind of activity. In contrast with the results obtained for tomato (Sgherri et al., 2007a, 2008), in basil γ-tocopherol prevailed over α-tocopherol, which was recovered in amounts 4- to 10-fold lower than those in tomato berries. Changes in the relative proportions of α- and γ-tocopherol affected the nutritional value of basil grown under different conditions (Fig. 5A) since, although γ-tocopherol has weaker antioxidant properties than has α-tocopherol, it is considered an especially promising "other" vitamin E for human health, being able to trap peroxynitrite formed during inflammation (Sgherri et al., 2007a).

Lipoic acid is unique, among antioxidant molecules, because it retains protective functions in both reduced (dihydrolipoic acid, DHLA) and oxidised forms (lipoic acid, LA) and because, due to its solubility in both water and lipid phases, it connects the activity of antioxidants in the cell membrane (tocopherols) with antioxidants in the cytoplasm (AsA and glutathione), strengthening the antioxidant network (Navari-Izzo, Quartacci, & Sgherri, 2002). The decrease in DHLA, the more effective one, under soil cultivation (35S), might be responsible, at least in part, for the decrease in antioxidant activity of both aqueous and lipid extracts in comparison with 35H. The fact that leaves of basil grown in hydroponics are endowed with levels of DHLA 3-fold higher than those from plants cultivated in soil, highlights a further advantage of hydroponic cultivation: a way to enrich plants, at least basil, with lipoic acid. In fact, the LA/DHLA redox couple approaches the ideal, being considered the "universal antioxidant" and being used for detoxification of heavy metal poisoning, for possible application in atherosclerosis, to counteract side effects of diabetes and oxidative stress, and for exerting a protective effect against the formation of cataracts (Navari-Izzo et al., 2002 and literature cited therein; D'Amico, Navari-Izzo, Sgherri & Izzo, 2004). Lipoic acid has been listed among antioxidants in potatoes; it has also been found in wheat leaves and roots (Sgherri et al., 2002), in tomato berries (Sgherri et al., 2007a, 2008), and this is the first time that its presence is demonstrated in basil (Fig. 5B).

The decrease in antioxidant activity of aqueous extract (Figs. 2A and 3A) was likely due to the decrease in AsA content (Fig. 4A). The biologically active form of vitamin C in antioxidative metabolism is its reduced form (AsA), and the percentage of AsA (on the total) remained unchanged in both 20H and 35H. Hydroponic cultivation optimises growth conditions, reducing the possibility of plants undergoing oxidative stress following environmental changes. AsA% decrease under soil cultivation (35S), compared to hydroponics (Fig. 4A), might be indicative of a greater oxidation of AsA in 35S, making leaves of basil grown in hydroponics and, in particular, 20H especially enriched in AsA. 20H was also particularly endowed with phenolic compounds compared to 35H and 35S (Fig. 4B) and the decrease in phenolic content in 35H compared to 20H could explain, in part, the reduction in the aqueous antioxidant activity from 20H to 35H (Figs. 2A and 3A). Anyway, the contribution to antioxidant activity depends on the composition of phenols (Jayasinghe, Gotoh, Aoki, & Wada, 2003) and phenolic acid compositions were completely different in the three samples (Table 1). In fact, the parent phenolic acids, benzoic and cinnamic acids, have TEAC values of zero, demonstrating no antioxidant activity, whereas dihydroxylation and trihydroxylation of the phenol ring can progressively increase antioxidant activity of these compounds, antioxidant response achieved being dependent on the relative position of the hydroxyl groups (Miller & Rice-Evans, 1997). Increases in vanillic, chlorogenic and caffeic acids in 35S compared to 35H (Table 1) reflect what was previously observed in *Raphanus sativus* grown in copper excess (Sgherri et al., 2003), suggesting, once more, that hydroponic cultivation prevents abiotic oxidative stress. In fact, phenolic acids are synthesised by plants in response to physical injury, infection or other stresses and they are stored primarily in the apoplast or in the vacuole, strategically playing either a signalling or direct role in defence. Notwithstanding the fact that 35S contained the higher value of chlorogenic acid (Table 1), which according to Rice-Evans et al. (1996) appears to be more active as an antioxidant than the hydroxyl-derivatives of benzoic acid, such as *p*-hydroxybenzoic, vanillic and syringic acids, the level of rosmarinic acid in 35S was about half of that in 35H (Table 1). The scientific interest in rosmarinic acid is growing, due to its antimicrobial, antiviral, antiallergic and anti-inflammatory properties and this lower content, makes basil grown under soil cultivation (35S) less interesting from a nutraceutical point of view. The amount of total phenolics for Genovese basil (Fig. 4B) is in agreement with that previously found by Nguyen and Niemeyer (2008), even if the content of rosmarinic acid is lower. Generally, the solvent used in the extraction influences the recovery and, despite methanol being the best candidate for the extraction of antioxidants from *O. basilicum* (Jayasinghe et al., 2003), the methanolic extract from 35S showed levels no more than 3-fold higher (197 μg/g DW) than that of the aqueous extract (Table 1). Moreover, besides their recovery, due to the kind of extraction, species, organ and type of cultivation might also influence phenol accumulation, thus explaining the differences found in the literature (Table 1; Nguyen & Niemeyer, 2008).

In conclusion, hydroponic cultivation ("floating system") of basil, which begins to have practical applications in Tuscany (Italy), reduces the possibility of plant undergoing oxidative stress following environmental changes, and improves nutritional value. Antioxidant activities of both aqueous and lipid extracts, as well as the concentrations of AsA and DHLA, were higher in basil leaves grown in hydroponics (35H) than in those grown in soil (35S). In particular, basil addressed to the Genovese market (20H) showed higher contents of vitamin C (AsA), vitamin E (α- and γ-tocopherol), total phenols and rosmarinic acid, together with a higher antioxidant activity, than basil addressed to the Tuscany market (35H).

Acknowledgement

This paper was supported by The University of Pisa (Ricerca d'Ateneo 2006).

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