Food Chemistry 114 (2009) 765–770

Contents lists available at ScienceDirect

Food Chemistry

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

Polyphenol content and antiradical activity of *Cichorium intybus* L. from biodynamic and conventional farming

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ARTICLE INFO

Article history:
Received 10 June 2008
Received in revised form 14 August 2008
Accepted 7 October 2008

Keywords:
Cichorium intybus
Polyphenols
Antiradical activity
Biodynamic agriculture

ABSTRACT

Conventionally- and biodynamically-grown chicory (*Cichorium intybus* L.) was compared for its polyphenol content and antiradical activity. Two growing periods were analysed: in the first, the plants were subjected to severe water stress; in the second the stress was absent. The polyphenol content (Folin–Ciocalteu test) was higher in samples from the former than in the latter (about 650 and 420mg of gallic acid/100g fresh sample, respectively), and in any case did not differ between the two growing systems; antiradical activity for the second sampling was higher in the case of the biodynamic system. HPLC/DAD/MS analysis identified five hydroxycinnamic acids and eight flavonoids (quercetin, kaempferol, luteolin and apigenin glycosides) and indicated changes in hydroxycinnamic content in the four samplings which were greatest in the case of conventional farming. Biodynamic farming, like organic farming, allows the achievement of good results, with particular attention to environmental conditions.

1. Introduction

Biodynamic farming is similar in many ways to better-known organic agriculture; both use composting and cover cropping instead of mineral fertilising and ban pesticides, herbicides, hormones and other chemicals. The difference from organic agriculture, apart from philosophical and historical aspects, lies in the use of biodynamic preparations which contain specific herbs or minerals, treated or fermented with animal organs, water and/or soil. These preparations are applied in finely-diluted form (homoeopathically), generally as field sprays after dynamisation, i.e., agitated in a specific way for long periods. The different types and aims of biodynamic preparations have been described (Carpenter-Boggs, Kennedy, & Reganold, 2000; Reeve et al., 2005). The aim of biodynamic preparations is supposed to lie in the improvement of soil and crop quality (Reganold, 1995), even if not all results support such an assumption. Generally, biodynamic farming is regarded as a holistic method which involves a spiritual world-view known as anthroposophy. In biodynamic management, and overall in biodynamic preparations, a scientific approach is lacking, with few exceptions (Carpenter-Boggs, Kennedy et al., 2000; Carpenter-Boggs, Reganold, & Kennedy, 2000; Reeve et al., 2005; Reganold, 1995). However, biodynamic farming is widespread and is increasing worldwide; in Italy in 2007 more than 300 farms commercialised biodynamically-grown crops, a 2–3% increase compared to 2006 (DEMETER data).

It seems that biodynamic farming supports many benefits with regard to sustainability and soil quality (Mäder et al., 2002; Reganold, Palmer, Lockhart, & Macgregor, 1993), but differences between organic and biodynamic practices have not been discovered (Carpenter-Boggs, Reganold et al., 2000; Reeve et al., 2005), even if greater soil biological and microbial activity have been reported (Mäder et al., 2002). In terms of crop quality, the comparison is more difficult. Some studies concern grape quality: however, one study on wine grape quality showed no differences in leaf and grape analysis, and only in one year (the study lasted four years) was a higher content of polyphenols and anthocyanins found in biodynamically-cultivated grape with respect to organically-cultivated plants (Reeve et al., 2005). When comparing conventional and organic farming, a higher level of total phenols was found in organically-grown Marion berry, strawberry and corn (Asami, Hong, Barrett, & Mitchell, 2003), and a higher antioxidant potential in apple from biodynamic farming (Carbonaro, Mattera, Vicoli, & Cappelloni, 2000) was found. In the case of wheat grains from organic and conventional agriculture, no consistent differences in the metabolic profile (amino acids, stress markers, sugars and sugar alcohols, nucleotides, urea and vitamin B5) were found (Zörb, Langenkämper, Betsche, Niehaus, & Barsch, 2006), and no other differences were detected when grains from organic, biodynamic and conventional growing systems were compared (Langenkämper et al., 2006). In a recent study on lettuce, no significantly higher levels of phenolics were recorded in the organically-grown vegetable (Young et al., 2005).

The present study concerns biodynamic and conventionally-grown chicory (*Cichorium intybus* L.) with the aim of ascertaining

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doi:10.1016/j.foodchem.2008.10.010

whether biodynamic food, which may be eaten after minimal processing, exhibits different characteristics with respect to the conventional crop in terms of polyphenol content and antiradical activity. Differences in agronomic practices should affect the polyphenol content since these compounds are involved in plant stress control and their quantity should increase due to biotic stress caused by the lack of pesticide use (Young et al., 2005). Owing to the contradictory results reported in literature, we attempted to design a very simple experimental plot, made possible by the chosen leafy vegetable, i.e., chicory cv. Spadona, which is consumed fresh in Tuscany and characterised by a high polyphenol content (Heimler, Isolani, Vignolini, Tombelli, & Romani, 2007).

2. Experimental

2.1. Agricultural conditions and plant material

Cichorium intybus L., cv. Spadona was cultivated under biodynamic and conventional production systems in replicated plots in the experimental orchard of the Biodynamic Association of Tuscany, located in Florence, Italy. The design was composed of three blocks, each containing a conventional and biodynamic plot. In November 2006, six plants of chicory were planted in each plot and were treated with preparation 500 (biodynamic production) and Nitrophoska Gold (5 g each plant, conventional production); the main ingredient of preparation 500 is cow (*Bos taurus*) manure and it is used as field spray (Carpenter-Boggs, Kennedy et al., 2000; Carpenter-Boggs, Reganold et al., 2000). The first sampling was taken in May 2007. After sampling, weeds were removed and a further treatment with preparation 500 and Nitrophosca Gold was carried out. The final sampling was performed in June 2007. Samples for each plot consisted of the two external leaves cut from each plant.

2.2. Extraction of polyphenols

Samples were frozen in liquid nitrogen and stored at −80 °C before proceeding with the analysis. Frozen tissues were ground in a mortar with a pestle under liquid nitrogen. A quantity of 2 g of tissue was extracted in 30 ml of 70% ethanol (pH 3.2 by formic acid) overnight. This solution was used for the determination of antioxidant activity, total phenolic, flavonoid contents, Fe^{2+} chelating ability and for HPLC analysis. The extraction yield (95%) was controlled by the addition of 40 μl gallic acid (5.88 mM) as internal standard; gallic acid is not naturally present in the samples and exhibits a retention time which falls in an empty zone of the chromatogram. Each experiment was run at least three times; all data are mean values.

2.3. Antiradical activity

Free radical scavenging activity was evaluated with the DPPH· (1,1-diphenyl-2-picrylhydrazyl radical) assay. The antiradical capacity of the sample extracts was estimated according to the procedure reported by Brand-Williams and Cuvelier (1995) and slightly modified. Two millilitres of the sample solution, suitably diluted with ethanol, was added to 2 ml of an ethanol solution of DPPH· (0.0025 g/100 ml) and the mixture was kept at room temperature. After 20 min, the absorption was measured at 517 nm with a Lambda 25 spectrophotometer (Perkin–Elmer) versus ethanol as a blank. Each day, the absorption of the DPPH· solution was checked. The antiradical activity is expressed as IC_{50}, the antiradical dose required to cause a 50% inhibition. IC_{50} was calculated plotting the ratio: $(A_{blank} - A_{sample}/A_{blank}) \times 100$, where A_{blank} is the absorption of the DPPH· solution and A_{sample} is the absorption of the DPPH· solution after addition of the sample, against the concentration of the sample. IC_{50} is expressed as mg sample/mg DPPH·.

2.4. Total phenolic content

The total phenolic content was determined using the Folin–Ciocalteu method, described by Singleton, Orthofer, and Lamuela-Raventos (1999) and slightly modified according to Dewanto, Wu, Adom, and Liu (2002). To 125 μl of the suitably diluted sample extract, 0.5 ml of deionised water and 125 μl of the Folin–Ciocalteu reagent were added. The mixture was kept for 6 min and then 1.25 ml of a 7% aqueous Na_2CO_3 solution were added. The final volume was adjusted to 3 ml with water. After 90 min, the absorption was measured at 760 nm against water as a blank. The amount of total phenolics is expressed as gallic acid equivalents (GAE, mg gallic acid/100 g sample) through the calibration curve of gallic acid. The calibration curve ranged from 20 to 500 μg/ml (R^2 = 0.9969).

2.5. Total flavonoid content

The total flavonoid content was determined using a colorimetric method described by Dewanto et al. (2002) and slightly modified in our laboratory. To 0.25 ml of the suitably diluted sample, 75 μl of a 5% $NaNO_2$ solution, 0.150 ml of a freshly prepared 10% $AlCl_3$ solution, and 0.5 ml of 1 M NaOH solution were added. The final volume was adjusted to 2.5 ml with deionised water. The mixture was allowed to stand for 5 min and the absorption was measured at 510 nm against the same mixture, without the sample, as a blank. The amount of total flavonoids is expressed as (+)catechin equivalents (CE, mg (+)catechin/100 g sample) through the calibration curve of (+)catechin. The calibration curve ranged 10–500 μg/ml (R^2 = 0.9946).

2.6. HPLC/DAD analysis

Analyses of flavonols and hydroxycinnamic derivatives were carried out using a HP 1100L liquid chromatograph equipped with a diode array detector (DAD) and managed by a HP 9000 workstation (Agilent Technologies, Palo Alto, CA, USA). Analysis was effectuated over a 30-min period at a flow rate of 0.8 ml min^{-1} using a Varian Polaris™ C18-E (250 × 4.6 mm id, 5 μm) column operating at 27 °C using a linear solvent gradient system (Table 1). UV/Vis spectra were recorded in the 190–600 nm range and the chromatograms were acquired at 260, 280, 330 and 350 nm.

2.7. HPLC/MS analysis

Analyses were performed using a HP 1100L liquid chromatograph linked to a HP 1100 MSD mass spectrometer with an API/Electrospray interface (Agilent Technologies, Palo Alto, CA, USA). The mass spectrometer operating conditions were: gas temperature, 350 °C; nitrogen flow rate, 11.0 l min^{-1}; nebulizer pressure,

Table 1
Linear solvent gradient system used in HPLC–DAD and HPLC–MS analysis of polyphenols in salad samples. (Analysis was carried out during a 30-min period at flow rate of 0.8 ml min^{-1} using a Varian Polaris™ C18-E (250 × 4.6 mm id, 5 μm) column operating at 27 °C.)

Time (min)	H_2O/H^+%	CH_3CN%	Flow
0	95	5	0.8
10	86	14	0.8
12	86	14	0.8
16	75	25	0.8
28	25	75	0.8
30	0	100	0.8