Conventional, organic and biodynamic farming: differences in polyphenol content and antioxidant activity of Batavia lettuce

Daniela Heimler,a* Pamela Vignolini,b Paola Arfaioli,a Laura Isolani,a and Annalisa Romani,b

Abstract

BACKGROUND: Lactuca sativa L. ssp. acephala L., cv. Batavia red Mohican plants were cultivated under intensive conventional, organic and biodynamic farming and were analyzed for their polyphenol content and antiradical activity in order to demonstrate the influence of farming on yield, polyphenol content and antiradical activity.

RESULTS: The yield of plants from conventional farming was the highest (2.89 kg m⁻²), while polyphenol content, measured by spectrophotometry, of these plants was lower at \( P < 0.05 \) (1.36 mg g⁻¹) than the content of plants from organic and biodynamic farming (1.74 and 1.85 mg g⁻¹, respectively). The antiradical activity, measured by DPPH · assay, was positively correlated to flavonoid and hydroxycinnamic acid contents.

CONCLUSION: Flavonoid, hydroxycinnamic acid and anthocyan patterns were not affected by the type of cultivation, while quantitative differences were demonstrated and some differences were found between conventional farming and organic or biodynamic farming. The yield of conventionally grown salads was the highest.

© 2011 Society of Chemical Industry

Keywords: flavonoids; anthocyanins; antiradical activity; agronomic yield; biodynamic farming

INTRODUCTION

Food products of organic origin are believed to be healthier than those from conventional farming. The concept of healthfulness concerns two main aspects: the first is correlated to agronomic practices and to their effects on soil management and food security (absence of synthetic pesticides); the second to the compounds of plants food such as vitamins, minerals, fibres and phytochemicals. Among these last compounds polyphenols play an important role in exerting their positive activity on human health and contributing strongly to protection from oxidative stress and regulation of cellular processes such as inflammation.2

While for the first aspect, especially in terms of sustainability, soil fertility and lesser use of pesticides, organic farming offers more benefit than a conventional approach, the second aspect involves greater certainty since the content and profile of polyphenols are affected by several factors such as temperature, ultraviolet light, insect attack, pathogen infection and nutrient deficiency. However, assessment of food quality is a very important subject and many parameters are taken into account. The comparison of organically and conventionally grown food has been reviewed, as has the quality of plant products from organic agriculture. Polyphenol content and antioxidant activity have been taken into account in the case of apples, peaches, different kinds of salads, wine grapes, oranges, blackcurrants, plums, oat grains, marionberries, strawberries and corn. The results of such comparisons are not univocal, and in some cases debate has been reported in the literature, depending in large part on the many variables which determine the amount of polyphenols.

Biodynamic agriculture falls into the category of general organic agriculture, the main differences being in the use of biodynamic preparations for soil (preparation 500), plants (preparation 501) and compost (preparations 502–507). The agronomic system is based on a holistic approach of the whole farm and under this aspect the simple use of preparations could not completely defined as a biodynamic approach. However, from a scientific point of view the only way to compare results from different farming systems is to study the effect of preparations.

Even if some efforts have been made to explain the mechanism by which these preparations act, their effect on plant physiology, soil microbiology and compost characteristics is still not explained. Only a few papers compare the results obtained with biodynamic agriculture with those from conventional farming.
The aim of the present paper is to compare three different agricultural practices (i.e. intensive conventional, organic and biodynamic) on the basis of agronomic yields and from the standpoint of polyphenol (flavonoids, anthocyanins and hydroxycinnamic acids) content and antiradical activity in order to ascertain whether biodynamic practices could lead to variations with respect to the most general organic practice and whether either technique could lead to vegetables which may be more appreciated by consumers as ‘nutraceutical’ food.

**EXPERIMENTAL**

**Agricultural conditions and plant material**

The experimental field was in the area of the ‘Forschungsring für Biologisch-Dynamische Wirtschaftsweise’ of Darmstadt, Germany. The soil is sandy orthic luvisol (FAO classification), with 87% sand, 8% loam and 5% clay. Seedlings of Batavia lettuce (Lactuca sativa L. ssp. acephala L., cv. Batavia red Mohican) were employed in our experiment under the three farming methods. The experimental layout was a randomized block (4 × 1.50 m, 102 seedlings), with three replicates for each kind of cultivation; distances were 25 cm among rows and 30 cm on the row.

The amount of mineral fertilization corresponded to 125 kg ha⁻¹ for nitrogen, 134 kg ha⁻¹ for phosphorus and 14 kg ha⁻¹ for potassium. 7.5 kg composted manure was distributed in organic and biodynamic blocks; the manure contained 125 kg ha⁻¹ nitrogen, and the phosphorus and potassium percentages (0.3% and 0.8% respectively) were calculated from the dry matter. The plants were irrigated when needed using the same quantity for nitrogen, 134 kg ha⁻¹ for phosphorus and 14 kg ha⁻¹ for potassium.

Seven and 3% of the total amount of potassium were applied in the form of K₂O.

The amounts of mineral fertilization corresponded to 125 kg ha⁻¹ for nitrogen, 134 kg ha⁻¹ for phosphorus and 14 kg ha⁻¹ for potassium. 7.5 kg composted manure was distributed in organic and biodynamic blocks; the manure contained 125 kg ha⁻¹ nitrogen, and the phosphorus and potassium percentages (0.3% and 0.8% respectively) were calculated from the dry matter. The plants were irrigated when needed using the same quantity of water in all plots.

The dates and the treatments for each cultivation are reported in Table 1. During this period total rainfall was 75.4 mm and the mean temperature was 12.42 °C (7.2 °C minimum, 17.2 °C maximum).

Eight lettuce plants were picked in each plot and grouped into subsamples of two plants so that 36 samples of the whole plant were obtained.

**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 3:7 water-ethanol mixture (pH = 3.2 by formic acid) overnight. The extraction yield (95%) was controlled by the addition of 40 µL gallic acid (5.88 mmol L⁻¹) 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. Only in the case of anthocyan analysis, the extraction was carried out with 70% ethanol (pH = 2 by formic acid).

**Standards and solvents**

Rutin, chlorogenic acid, gallic acid, Folin–Ciocalteu reagent and DPPH (1,1-diphenyl-2-picrylhydrazyl radical) were purchased from Sigma-Aldrich (St Louis, MO, USA). Malvin-3-O-glucoside was purchased from Extrasynthese (Nord Genay, France).

All solvents used were of high-performance liquid chromatography (HPLC) grade purity (BDH Laboratory Supplies, Poole, UK).

**Antiradical activity**

Free radical scavenging activity was evaluated with the DPPH assay. The antiradical capacity of the sample extracts was estimated according to the procedure reported by Brand-Williams and Cuvelier, with slight modifications. Two milliliters of the sample solution, suitably diluted with ethanol, was added to 2 mL of an ethanol solution of DPPH (0.0025 g 100 mL⁻¹) and the mixture was kept at room temperature. After 20 min, the absorption was measured at 517 nm versus ethanol as a blank. Each day, the absorption of the DPPH solution was checked.

Antiradical activity was expressed as IC₅₀: the antiradical dose required to cause 50% inhibition. IC₅₀ was calculated by plotting the ratio (A_blank − A_sample/A_blank) × 100, where A_blank is the absorption of DPPH solution and A_sample is the absorption of DPPH solution after addition of the sample, against the concentration of the sample. Straight lines were obtained in each case, with R² changing from 0.8179 to 0.9981. IC₅₀ was expressed as mg sample mg⁻¹ DPPH. All spectrophotometric data were achieved using a Lambda 25 spectrophotometer (PerkinElmer, Waltham, MA, USA).

**Total phenolic content**

The total phenolic content was determined using the Folin–Ciocalteu method, described by Singleton et al. and slightly modified. To 125 µL of the suitably diluted sample extract, 0.5 mL of deionized water and 125 µL of the Folin–Ciocalteu reagent were added. After 6 min 1.25 mL of a 7% aqueous Na₂CO₃ solution was added to the mixture. 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 was expressed as gallic acid equivalents (GAE, mg gallic acid 100 g⁻¹ sample) through the calibration curve of gallic acid. The calibration curve ranged from 20 to 500 µg mL⁻¹ (r² = 0.9969).

**Total anthocyan content**

Anthocyan levels were estimated by means of spectrophotometric measurements from the extraction solution as A₅₃₀ = 0.24A653.
HPLC – diode array detection analysis (DAD)
Analyses of flavonols and hydroxycinnamic acids were carried out using an HP 1100 liquid chromatograph equipped with a diode array detector and managed by a HP 9000 workstation (Agilent Technologies, Palo Alto, CA, USA). Analysis was carried out during a 30 min period at a flow rate of 0.8 mL min⁻¹ using a Varian Polaris C18-E (250 × 4.6 mm i.d., 5 μm) column operating at 27 °C with a linear solvent gradient system. UV-visible spectra were recorded in the 190–600 nm range and chromatograms were acquired at 260, 280, 330 and 350 nm.

HPLC – mass spectrometry (MS)
Analyses were performed using an HP 1100 liquid chromatograph linked to an HP 1100 MSD mass spectrometer with an atmospheric pressure ionization/electrospray interface (Agilent Technologies). The mass spectrometer operating conditions were: gas temperature, 350 °C; nitrogen flow rate, 11.0 L min⁻¹; nebulizer pressure, 40 psi; quadrupole temperature, 100 °C; and capillary voltage, 4000 V. The mass spectrometer was operated in negative mode at 120 eV.

Identification and quantification of individual polyphenols
Identification of individual polyphenolic compounds was directly performed by HPLC/DAD using a five-point regression curve ($r^2 \geq 0.998$) in the range 0–30 μg on the basis of standards. In particular, flavonols were determined at 350 nm using rutin as reference compound. Hydroxycinnamic derivatives were determined at 330 nm using chlorogenic acid as reference compound. In all cases, actual concentrations of the derivatives were calculated after applying corrections for differences in molecular weight. Three samples were collected from each site so as to express the analytical results as an average with its standard deviation.

Statistical analysis
Statistical analysis was performed using PASW Statics, version 18, with the general linear model. Data were subjected to analysis of variance (ANOVA) and to Tuckey’s test. Differences at $P < 0.05$ were indicated by lower case letters, while differences at $P < 0.01$ were indicated by upper case letters. The t-test has been used for the analysis of some data. All data are mean values of three determinations.

RESULTS AND DISCUSSION
Figure 1 reports the average head weights of Batavia at three different sampling dates. The weights of the conventionally grown lettuce were always the highest and were also significantly different ($P < 0.001$) from both organic and biodynamic lettuce heads, while latter treatments significantly ($P < 0.05$) differ only for the last sampling date (21 July). Also in the case of organically grown apples, their weight was lower than that of conventionally grown fruits, while the opposite is reported in the case of plums. The yield of conventionally grown lettuce was therefore higher than that of the other two groups (2.89 kg m⁻² with respect to 2.30 and 2.37 kg m⁻² for organic and biodynamic farming for the last sampling). It should be noted that water content (about 93%) did not significantly change among the three farming systems.

The amounts, as determined by spectrophotometric analysis, of polyphenols and total anthocyanins under the three farming systems are reported in Table 2. Both anthocyan and polyphenol contents were significantly lower under conventional than under biodynamic farming. The amount of polyphenols in biodynamically grown lettuce was about 36% higher than in conventionally grown product and its amount (as gallic acid) in the latter, notwithstanding its higher yield, was 3.93 g m⁻²; for organic and biodynamic lettuce the values were 4.00 and 4.38 g m⁻² respectively. Similar results were obtained in the case of marionberries and strawberries. In a previous paper on organically grown lettuce the total amount of polyphenols was lower (from two to five times) than the values found in this research and no differences between organic and conventional cultivation have been found in individual and total phenolic levels. The discrepancy between these data can be ascribed to the different cultivars of seedlings and to the environmental conditions, which are also responsible for the expression of polyphenols.

Our trend is similar to that found in tomatoes, where a 10-year study showed higher flavonoid contents under organic management practices with respect to a conventional approach. In a previous study on Cichorium intybus no differences were found between conventional and biodynamic cultivations in terms of polyphenol content.

Anthocyan amount (Table 2) in conventionally grown Batavia lettuce was the lowest, also taking into account the agronomic yields (2.60 g m⁻²), while for lettuce from organic and biodynamic farming it was calculated as 3.28 and 4.43 g m⁻² respectively; this
trend is similar to what found in the case of strawberries. In the case of Syrah grapes grown under organic agricultural practices, a lower anthocyan content was achieved, which was ascribed to the use of pesticides in conventional farming. Organic farming tends to increase environmental stress and therefore an increase in polyphenol content can be explained on this basis.

With regard to antiradical activity, IC₅₀ values are not significantly different when conventional lettuce is compared to the organic and/or biodynamic produce. However, if the t-test is performed significant differences (P < 0.05) are found when conventional lettuce is compared to the organic and/or biodynamic produce, while the latter two groups do not differ between them.

The kinetic curves for the three farming systems (conventional, organic and biodynamic) were similar. The exponential model which describes the three curves (R² ≥ 0.9451) is

\[ \ln[DPPH] = at + b \]

indicating that the antiradical mechanism is the same in the three cases. However, no relationship could be found between antiradical activity and polyphenol or anthocyan contents, similarly to what observed in the case of basil (Ocimum basilicum L.), where antioxidant activity did not correlate with total polyphenol content, while the role of anthocyanins in plant protection from oxidative stress seems related to their ability to attenuate visible light and reduce excitation pressure.

Identification of individual polyphenols was carried out using HPLC-DAD-MS; a chromatogram, acquired at 350 nm, of a lettuce extract is reported in Fig. 2. From a qualitative point of view, quercetin and luteolin derivatives, and caffeic acid derivatives were identified. In particular, among flavonoids we identified luteolin glucuronide and quercetin malonylglucoside according to Heimler et al. Chlorogenic acid was also found and identified by comparison and combination of retention time, UV-visible and mass spectra with those of authentic standards.

The amounts of flavonoids and hydroxycinnamic acids as obtained from HPLC data are reported in Table 3. In all cases biodynamic and organic farming led to the highest amount of polyphenols. The total polyphenol content is comparable to that reported by Heimler et al. for different lettuce varieties. It can be pointed out that flavonoids are the main compounds in the three different samples (Table 3), and quercetin malonyl glucoside is always the most abundant compound (Fig. 3).

Both flavonoids and hydroxycinnamic acid contents correlate with the antiradical activity; also total polyphenols (from HPLC data) exhibit quite a good correlation with antiradical activity. In all cases R² values are between 0.799 and 0.928. Total phenolic content, measured with the Folin–Ciocalteu method, seems a good indicator of ABTS and ORAC scavenging capacities, while flavonoids and hydroxycinnamic acids are correlated to DPPH scavenging capacities, giving rise to a positive correlation (the higher the polyphenol amount, the greater the antiradical activity).

Figs 3 and 4 present the different trends for individual flavonoids and hydroxycinnamic acids. It is possible to note that the differences among the three cultivation methods involve only some quantitative aspects since the qualitative patterns do not change. In fact, the relative amounts of individual flavonoids and hydroxycinnamic acids do not change with the cultivation method.

In conclusion, the present study demonstrates that under the same conditions of climate, temperature, water stress and plant material, when comparing conventional, organic and biodynamic cultivation of Lactuca sativa L. ssp. acephala L., cv. Batavia red Mohican plants, the yield in the conventional treatment was highest, while the amount of polyphenols (see Table 2) was significantly lower than in biodynamic farming, with all the other parameters (amount of individual polyphenols, total anthocyanins) confirming this trend. As regards the differences between organic and biodynamic farming, no significant values were found.

If we regard polyphenol and hydroxycinnamic acid contents as ‘nutraceutical’ components of food, it should be underlined that in this case higher amounts were obtained with biodynamic and organic farming. The reason why this occurs may be associated with stress conditions in organic and biodynamic farming and/or to a different microbe environment. The differences between organic and biodynamic farming systems are not firmly stated in this case; applying the t-test instead of ANOVA, however,

![Fig. 2. Chromatographic profiles acquired by HPLC/DAD (350 nm) of the hydroalcoholic (ethanol: water 70:30, pH 3.2) extract of a lettuce sample. Identified compounds: 1, caffeic derivative; 2, caffeoylquinic derivative; 3, chlorogenic acid; 4, caffeic derivative; 5, flavonoid; 6, quercetin derivative; 7, luteolin glucuronide; 8, quercetin malonyl glucoside; 9, caffeic derivative; 10, quercetin acetyl glucoside; 11, caffeic derivative.](image)

![Fig. 3. Amount of individual flavonoids (mg g⁻¹). For each compound, there is a bar for conventional (C), organic (O) and biodynamic (B) farming. Numbers as in Fig. 2. Error bars describe the standard deviation of replicates (n = 12).](image)

| Table 3. Amount of flavonoids, hydroxycinnamic acid and total polyphenols (mg g⁻¹ sample) from HPLC data |
|---------------------------------|-----------------|-----------------|-----------------|
| Farming                        | Flavonoids      | Hydroxycinnamic | Total polyphenols |
| Conventional                   | 1.09A           | 0.67A           | 1.76a           |
| Organic                        | 1.23AB          | 0.84AB          | 2.07a           |
| Biodynamic                     | 1.39B           | 0.97B           | 2.36a           |

Upper-case letters indicate a significance level <0.01. All data refer to fresh weight.
the differences between organic and biodynamic farming are significant at $P < 0.05$ and $P < 0.001$ in the case of anthocyan and hydroxyccinamic acid contents and this aspect could indicate an influence of biodynamic practice on secondary metabolites which has not previously been shown.

ACKNOWLEDGEMENT

This research was supported by funds from the Ente Cassa di Risparmio di Firenze, which contributed to the acquisition of part of the instrumentation used for this work. We express our thanks to Dr Uwe Geier and the staff of Forschungsring für Biologisch-Dynamische Wirtschaftsweise of Darmstadt, Germany, for providing plant material and to Elisabetta Posth for her technical assistance.

REFERENCES


