Research Article

SCI

Received: 23 February 2011 Revised: 5 July 2011 Accepted: 17 July 2011 Published online in Wiley Online Library: 20 September 2011

(wileyonlinelibrary.com) DOI 10.1002/jsfa.4605

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.

Keywords: flavonoids; anthocyans; 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[1] 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,[3] the second aspect involves greater uncertainty since the content and profile of polyphenols are affected by several factors such as temperature, ultraviolet light, insect attack, pathogen infection and nutrient deficiency[4]. However, assessment of food quality is a very important subject and many parameters are taken into account.[5] The comparison of organically and conventionally grown food has been reviewed,[6] as has the quality of plant products from organic agriculture.[7] Polyphenol content and antioxidant activity have been taken into account in the case of apples,[8,9] peaches and pears,[10] different kinds of salads,[4] wine grapes,[11] oranges,[12] blackcurrants,[13] plums,[14] oat grains,[15] marionberries, strawberries and corn,[16] and tomatoes.[17] The results of such comparisons are not univocal, and in some cases debate has been reported in the literature,[18,19] 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 be 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.[20]

Even if some efforts have been made to explain the mechanism by which these preparations act,[21–23] their effect on plant physiology, soil microbiology and compost characteristics is still not explained.[20] Only a few papers compare the results obtained with biodynamic agriculture with those from conventional farming.[11,24,25]

* Correspondence to: Daniela Heimler, Dipartimento di Scienze delle Produzioni Vegetali, del Suolo e dell'Ambiente Agroforestale, University of Florence, Piazzale delle Cascine 18, 50144 Florence, Italy. E-mail: daniela.heimler@unifi.it

a Dipartimento di Scienze delle Produzioni Vegetali, del Suolo e dell'Ambiente Agroforestale, University of Florence, 50144 Florence, Italy

b Dipartimento di Scienze Farmaceutiche, University of Florence, 50019 Sesto Fiorentino, Florence, Italy

Table 1. Treatments and application dates for the three farming systems

Date	Conventional plots	Organic plots	Biodynamic plots
14 May 2008		Treatment with composted manure	Treatment with biodynamic composted manure and field preparation 500
29 May 2008	Seedlings transplanted and mineral fertilization (27% N, 18% P_2O_5 and 30% K_2O)	Seedlings transplanted	Seedlings transplanted
6 June 2008			Field preparation 501
13 June 2008			Field preparation 500
2 July 2008			Field preparation 501
11 July 2008	First sampling	First sampling	First sampling
14 July 2008	Second sampling	Second sampling	Second sampling
21 July 2008	Harvest	Harvest	Harvest

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, anthocyans 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 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- picrylhydrazil radical) were purchased from Sigma-Aldrich (St Louis, MO, USA). Malvin-3-*O*-glucoside was purchased from Extrashynthese (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,[26] with slight modifications. 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^{-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 IC_{50}: the antiradical dose required to cause 50% inhibition. IC_{50} was calculated by plotting the ratio $(A_{blank} - A_{sample}/A_{blank}) \times 100$, 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 R^2 changing from 0.8179 to 0.9981. IC_{50} 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.*[27] and slightly modified.[28] 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 Na_2CO_3 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} ($r^2 = 0.9969$).

Total anthocyan content

Anthocyan levels were estimated by means of spectrophotometric measurements from the extraction solution as $A_{530} - 0.24A653$.[29]

HPLC–diode array detection analysis (DAD)

Analyses of flavonols and hydroxycinnamic acids were carried out using an HP 1100L 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.[30] 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 1100L 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 polyphenols was carried out using their retention times and both spectroscopic and mass spectrometric data. Quantification 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,[10] while the opposite is reported in the case of plums.[14] 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.

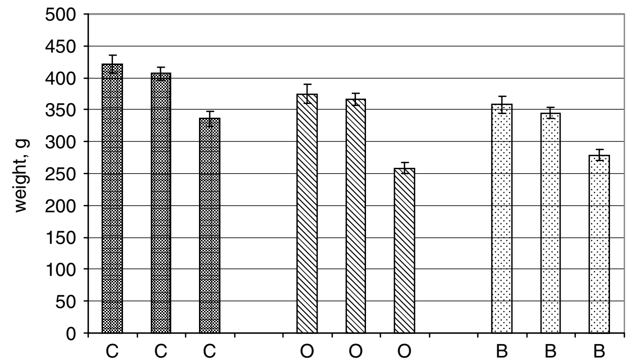

Figure 1. Average weight of a head of lettuce at three samplings dates (11 July, first bar; 14 July, second bar; 21 July 2008, third bar). The data are divided according to conventional trial (C), organic trial (O) and biodynamic trial (B). Error bars describe the standard deviation of replicates ($n = 20$).

Table 2. Amount of polyphenols, anthocyans and antiradical activity expressed as IC_{50}, i.e. the amount of sample (mg) which inhibited the activity of 1 mg DPPH ·

Farming	Polyphenols as gallic acid (mg g^{-1} sample)	Anthocyans as malvin-3-*O*-glucoside (mg g^{-1} sample)	DPPH · IC_{50} (mg sample mg^{-1} DPPH)
Conventional	1.36a	0.90A	238.80a
Organic	1.74ab	1.43AB	199.48a
Biodynamic	1.85b	1.87B	197.95a

Upper-case letters indicate a significance level <0.01; lower-case letters indicate a significance level <0.05. All data refer to fresh weight.

The amounts, as determined by spectrophotometric analysis, of polyphenols and total anthocyans 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.[16] In a previous paper[4] 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.[17] In a previous study on *Cichorium intybus*[24] 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

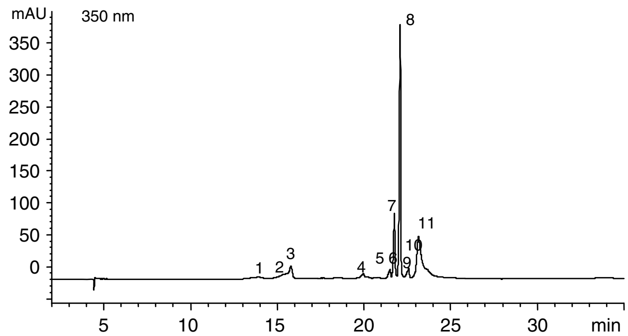

Figure 2. Chromatographic profiles acquired by HPLC/DAD (350 nm) of the hydroalcoholic (ethanol : water 70 : 30, pH 3.2) extract of a lettuce sample. Identified compounds: 1, caffeic derivative; 2, caffeoylquinic derivative; 3, chlorogenic acid; 4, caffeic derivative; 5, flavonoid; 6, quercetin derivative;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

Table 3. Amount of flavonoids, hydroxycinnamic acid and total polyphenols (mg g^{-1} sample) from HPLC data

Farming	Flavonoids	Hydroxycinnamic acids	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.

trend is similar to what found in the case of strawberries.[31] 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.[32] Organic farming tends to increase environmental stress and therefore an increase in polyphenol content can be explained on this basis.[33]

With regard to antiradical activity, IC_{50} 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 ($R^2 \geq 0.9451$) is

$$\ln[\text{DPPH}\cdot] = at + b$$

indicating that the antiradical mechanism is the same in the three cases.[30] 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,[34] while the role of anthocyans in plant protection from oxidative stress seems related to their ability to attenuate visible light and reduce excitation pressure.[35]

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 flavonols we identified luteolin glucuronide and quercetin malonylglucoside according to Heimler *et al.*[30] 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.*[30] 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).

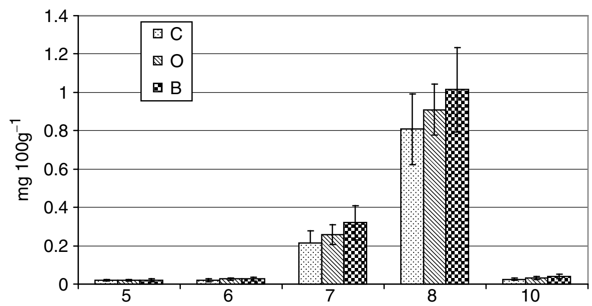

Figure 3. Amount of individual flavonoids (mg g^{-1}). For each compound, there is a bar for conventional (C), organic (O) and biodynamic (B) farming. Numbers as in Fig. 2. Error bars describe the standard deviation of replicates ($n = 12$).

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 R^2 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,[36] 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 anthocyans) 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.[22] The differences between organic and biodynamic farming systems are not firmly stated in this case; applying the *t*-test instead of ANOVA, however,

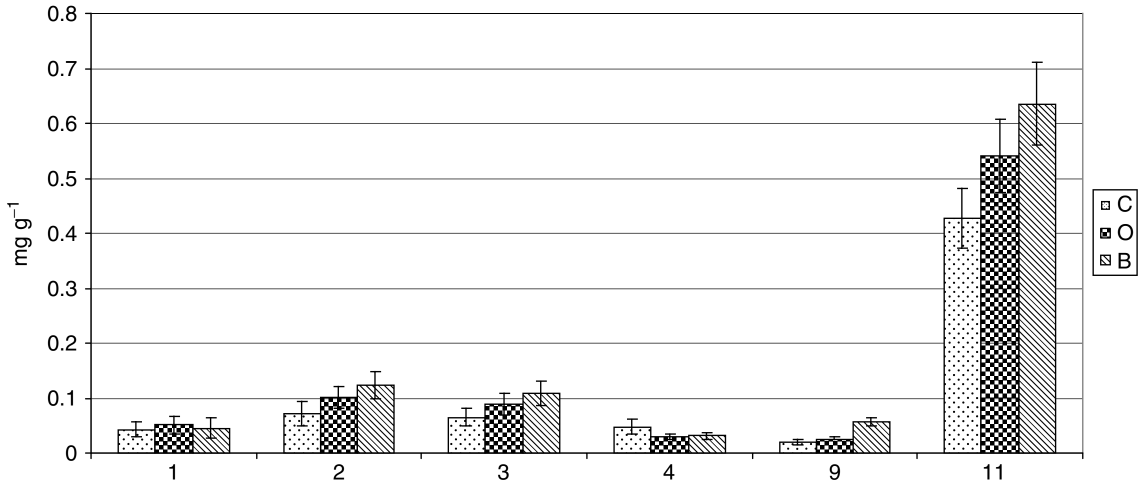

Figure 4. Amounts of individual hydroxycinnamic acids (mg g^{-1}). For each compound, there is a bar for conventional (C), organic (O) and biodynamic (B) farming. Numbers as in Fig. 2. Error bars describe the standard deviation of replicates ($n = 12$).

the differences between organic and biodynamic farming are significant at $P < 0.05$ and $P < 0.001$ in the case of anthocyan and hydroxycinnamic 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

1 Manach C, Scalbert A, Morand C, Remsey C and Jimenez L, Polyphenols: food sources and bioavailability. *Am J Clin Nutr* **79**:727–747 (2004).
2 Stevenson DE and Hurst RD, Polyphenolic phytochemicals: just antioxidant or much more? *Cell Mol Life Sci* **64**:2900–2916 (2007).
3 Pimentel D, Hepperly P, Hanson J, Doubs D and Seidel R, Environmental, energetic, and economic comparisons of organic and conventional farming systems. *BioScience* **55**:573–582 (2005).
4 Young JE, Zhao X, Carey EE, Welti R, Yang S-S and Wang W, Phytochemical phenolics in organically grown vegetables. *Mol Nutr Food Res* **49**:1136–1142 (2005).
5 Hounsome N, Hounsome B and Edward-Jones G, Plant metabolites and nutritional quality of vegetables. *J Food Sci* **73**:R48–R65 (2008).
6 Woese K, Lange D, Boess C and Bögl KW, A comparison of organically and conventionally grown foods: results of a review of the relevant literature. *J Sci Food Agric* **74**:281–293 (1997).
7 Rembialkowska E, Quality of plant products from organic agriculture. *J Sci Food Agric* **87**:2757–2762 (2007).
8 Valavanidis A, Vlachogianni T, Psomas A, Zovoili A and Siatis V, Polyphenolic profile and antioxidant activity of five apple cultivars grown under organic and conventional agricultural practice. *Int J Food Sci Technol* **44**:1167–1175 (2009).
9 Stracke BA, Rüfer CE, Weibel FP, Bub A and Watzl, B, Three-year comparison of the polyphenol contents and antioxidant capacities in organically and conventionally produced apples (*Malus domestica* Bork. Cultivar 'Golden delicious'). *J Agric Food Chem* **57**:4598–4605 (2009).
10 Carbonaro M, Mattera M, Nicoli S, Bergamo P and Cappelloni M, Modulation of antioxidant compounds in organic vs. conventional fruits (peach, *Prunus persica* L., and pear, *Pyrus communis* L.). *J Agric Food Chem* **50**:5458–5462 (2002).
11 Reeve JR, Carpenter-Boggs L, Reganold JP, York AL, McGourthy G and McCloskey LP, Soil and winegrape quality in biodynamically and organically managed vineyards. *Am J Enol Viitic* **46**:367–376 (2005).
12 Tarozzi A, Hrelia S, Angeloni C, Morroni F, Biagi P, Guardigli M, *et al*, Antioxidant effectiveness of organically and non-organically grown red oranges in cell culture systems. *Eur J Nutr* **45**:152–158 (2006).
13 Anttonen MJ and Karjalainen RO, High-performance liquid chromatography analysis of black current (*Ribes nigrum* L.) fruit phenolics grown either conventionally or organically. *J Agric Food Chem* **54**:7530–7538 (2006).
14 Lombardi-Boccia G, Lucarini M, Lanzi S, Aguzzi A and Cappelloni M, Nutrients and antioxidant molecules in yellow plums (*Prunus domestica* L.) from conventional and organic production: a comparative study. *J Agric Food Chem* **52**:90–94 (2004).
15 Dimberg LH, Gissen C and Nilsson J, Phenolic compounds in oat grains (*Avena sativa* L.) grown in conventional and organic systems. *Ambio* **34**:331–337 (2005).
16 Asami DK, Hong Y-J, Barrett DM and Mitchell AE, Comparison of total phenolic and ascorbic acid content of freeze-dried and air-dried marionberry, strawberry, and corn using conventional, organic and sustainable agricultural practice. *J Agric Food Chem* **51**:1237–1241 (2003).
17 Mitchell AE, Hong Y-J, Koh E, Barret, DM, Bryant DE, Denison RF *et al*, Ten-year comparison of the influence of organic and conventional crop management practices on the content of flavonoids in tomatoes. *J Agric Food Chem* **55**:6154–6159 (2007).
18 Felson AS and Rosen JD, Comment on comparison of the total phenolic and ascorbic acid content of freeze-dried and air-dried marionberry, strawberry, and corn using conventional, organic and sustainable agricultural practices. *J Agric Food Chem* **52**:146–149 (2004).
19 Mitchell AE and Barrett DM, Rebuttal on comparison of the total phenolic and ascorbic acid content of freeze-dried and air-dried marionberry, strawberry, and corn using conventional, organic and sustainable agricultural practices. *J Agric Food Chem* **52**:150–152 (2004).
20 Turinek M, Grobelnik-Mlakar S, Bavec M and Bavec F, Biodynamic agriculture research progress and priorities. *Renew Agric Food Syst* **24**:146–154 (2009).
21 Carpenter-Boggs L, Kennedy AC and Reganold JP, Organic and biodynamic management: effect on soil biology. *Soil Sci Am J* **64**:1651–1658 (2002).
22 Carpenter-Boggs L, Reganold JP and Kennedy AC, Effects of biodynamic preparations on compost development. *Biol Agric Hortic* **17**:313–328 (2000).

23 Carpenter-Boggs L, Reganold JP and Kennedy AC, Biodynamic preparations: short-term effects on crops, soils and weed populations. *Am J Alternat Agric* **15**:110–118 (2000).
24 Heimler D, Isolani L, Vignolini P and Romani A, Polyphenol content and antiradical activity of *Cichorium intybus* L. from biodynamic and conventional farming. *Food Chem* **114**:765–770 (2009).
25 Tung LD and Fernandez PG, Soybean under organic, biodynamic and chemical production at the Mekong Delta, Vietnam. *Philip J Crop Sci* **32**:49–61 (2007).
26 Brand-Williams W and Cuvelier ME, Use of a free radical method to evaluate the antioxidant activity. *Lebens-Wiss Technol* **28**:25–30 (1995).
27 Singleton VL, Orthofer R and Lamuela-Raventos RM, Analysis of total phenols and other oxidation substrates and antioxidants by means of the Folin–Ciocalteu reagent. *Meth Enzymol* **299**:152–178 (1999).
28 Dewanto V, Wu X, Adom KK and Liu RH, Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. *J Agric Food Chem* **50**:3010–3014 (2002).
29 Gould KS, Markham KR, Smith RH and Goris JJ, Functional role of anthocyanins in the leaves of *Quintia serrata* A. Cunn. *J Exp Bot* **51**:1107–1115 (2000).
30 Heimler D, Isolani L, Vignolini P, Tombelli S and Romani A, Polyphenol content and antioxidant activity in some species of freshly consumed salads. *J Agric Food Chem* **55**:1724–1729 (2007).
31 D'Evoli L, Tarozzi A, Hrelia P, Lucarini M, Gabrielli F, Franco F, *et al*, Influence of cultivation system on bioactive molecule synthesis in strawberries: spin-off on antioxidant and antiproliferative activity. *J Food Sci* **75**:C94–C99 (2010).
32 Abert Vian M, Tomao V, Coulomb PO, Lacome JM and Dangles O, Comparison of the anthocyan composition during ripening of Syrah grapes grown using organic or conventional agricultural practices. *J Agric Food Chem* **54**:5230–5235 (2006).
33 Young JE, Zhao X, Carey EE, Welti R, Yang SS and Wang W, Phytochemical phenolics in organically grown vegetables. *Mol Nutr Food Res* **49**:1136–1142 (2005).
34 Nguyen PM and Niemeyer ED, Effects of nitrogen fertilization on the phenolic composition and antioxidant properties of basil (*Ocimum basilicum* L.). *J Agric Food Chem* **56**:8685–8691 (2008).
35 Steyn WJ, Wand SJE, Holcroft DM and Jacobs G, Anthocyanins in vegetative tissues: a proposed unified function in photoprotection. *New Phytol* **155**:349–361 (2002).
36 Moore J, Liu JG, Zhou K and Yu L, Effects of genotype and environment on the antioxidant properties of hard winter wheat bran. *J Agric Food Chem* **54**:5312–5322 (2006).

