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Chemical and sensory characterisation of Sangiovese red wines: Comparison between biodynamic and organic management

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ABSTRACT

The effects of biodynamic production practices on composition and sensory attributes of Sangiovese wines were examined for 2 years (2009 and 2010) in a vineyard that was converted from organic (ORG) to biodynamic (BDN) viticulture. During the first year (2009), the BDN wines were characterised by low alcohol strength, colour intensity, total polyphenols, monomeric anthocyanins and catechin. Conversely, the second year BDN wines differed from the organic wines in terms of total polyphenols and phenolic compounds, including polymeric pigments, co-pigmentation, tannins and iron-reactive polyphenols. The effect of management practices, harvest and their interaction was analysed for each compound. Positive interaction was observed for total acidity, volatile acidity, cyanidin-3-glucoside, protocatechuic acid, (+)-catechin, quercetin and *trans*-resveratrol.

ORG wine initially showed a more complex aroma profile; however, the differences were almost indistinguishable during the second year. Trained panellists highlighted differences in colour intensity between ORG and BDN wines although no preference was found by consumers. The concentrations of ochratoxin A and biogenic amines were far below the health-hazardous threshold.

1. Introduction

Wines produced using an environmentally sustainable approach, such as organic (Mann, Ferjani, & Reissig, 2012) and biodynamic practices (Meunier, 2001; Preston, 2008; Zucca, Smith, & Mitry, 2009) have enjoyed increasing popularity due to growing demands for healthy products.

In particular, biodynamic agriculture differs from traditional organic management, primarily in the use of specific fermented *preparations* proposed by Rudolf Steiner (1861–1925) which are claimed to stimulate the soil nutrient cycle, and enforce photosynthesis and optimal evolution of compost, enhancing both soil and crop quality (Koepf, Schaumann, & Haccius, 2001). Biodynamic management is considered to induce beneficial environmental effects on the energetic efficiency of sustainable agro-ecosystems (Turinek, Grobelnik-Mlakar, Bavec, & Bavec, 2009).

The Steiner *preparations* can improve the vegetative-reproductive balance of plants, increasing sugar, total polyphenol and anthocyanin concentrations of grapes (Reeve et al., 2005). Although a recent study demonstrated that ^{1}H-NMR was able to discriminate between red wines from organic and biodynamic grapes (Laghi, Versari, Marcolini, & Parpinello, 2014), very little information is available on the characteristics of biodynamic grape and wine (Plahuta & Raspor, 2007; Ross, Weller, Blue, & Reganold, 2009; Tassoni, Tango, & Ferri, 2013).

The satisfactory discrimination of organic wines from conventional wines (>73%), based on multiparametric mid-infrared signals related to wine composition (Cozzolino, Holdstock, Dambergs, Cynkar, & Smith, 2009), highest amounts of polyphenolic compounds, antioxidant activity (Miceli, Negro, Tommasi, & De Leo, 2003) and *trans*-resveratrol (Tinttunen & Lehtonen, 2001), has been reported. However, the difference between organic and conventional grape, must and wine was not always significant in terms of physicochemical and sensory characteristics (Mulero, Pardo, & Zafrilla, 2009; Mulero, Zafrilla, Cayuela, Martinez-Cacha, & Pardo, 2011) and controversial results were found between conventional and organic wines in terms of microbial metabolites, such as biogenic amines (Kalkan Yildirim, Üren, & Yücel, 2007; Yañez, Saavedra, Martínez, Córdova, & Ganga, 2012) and ochratoxin A (Miceli et al., 2003; Plahuta & Raspor, 2007; Ponsone, Combina, Dalcero, & Chulze, 2007). Trained panellists found that the use of

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selected yeasts during the fermentation of organic grapes improved the sensory quality of wines compared to the autochthonous (Callejon et al., 2010). In the wine industry, there are a number of different eco-labels related to organic and biodynamic certification that are only partially recognised and understood by consumers (Delmas & Grant, 2014). The European Community recently enacted a regulation (EC, 2012) which establishes that "organic wines" have to be produced with organic grape only (EC, 2007); the use of sulphur dioxide must be limited during the vinification process and storage, and some practices are restricted or prohibited. By contrast, there is a lack of official European regulation for biodynamic viticulture and winemaking; thus the producers interested in this sustainable approach must refer to protocols proposed by private organizations which encourage spontaneous fermentation, instead of the use of commercial selected yeasts. According to these protocols 2 years of conversion, starting from organic viticulture, are needed to achieve a biodynamically certifiable management.

The present study was designed to determine the effect of biodynamic *preparations* on the chemical and sensory attributes of Sangiovese red wines, the main autochthonous grape variety grown in Italy.

2. Materials and methods

2.1. Grape management

The Sangiovese red grapes, clone FEDIT 30 ESAVE, were harvested for two seasons (2009–2010), following the conversion of an organically maintained vineyard to biodynamic practices. The vineyard, (ca. 1 ha) is located in Tebano (Ravenna, Italy). The vineyard had vine by row spacing of 1 × 2.8 m, corresponding to 3571 spur-pruned vines per ha. Starting in 2007, the vineyard was managed as organic (ORG) in accordance with Reg. EC 834/2007 (EC., 2007); then, in 2009, about 50% of the vineyard was dedicated to biodynamic management (BDN), using the following '*preparations*' during the vegetative growth: soil application of cow manure (500; 100 g/ha) and fladen (cow manure enriched with basalt powder and eggshell; 100 g/ha), foliar application of finely ground quartz powder (501; 5 g/ha) (Spaccini, Mazzei, Squartini, Giannattasio, & Piccolo, 2012), soil application of 500 K (100 g/ha). In 2010, trunk paste (130 kg/ha), a mixture of fresh cow manure, horsetail and stinging nettle infusion, sand, bentonite and water, was applied to the trunks.

As the aim of the trial was to evaluate the effect of biodynamic preparations, no shoot or bunch thinning or other canopy management was performed on the vines.

2.2. Winemaking protocol

Although the vinifications were performed before the final approval of the Reg. EC 203/2012, the winemaking protocol used during the trial suited the requisites of the EU regulation in terms of organic wine production. During each harvest (2009 and 2010), two vinifications, for both viticultural managements, were set up (Organic: ORG_1 and ORG_2; Biodynamic: BDN_1 and BDN_2), each of them with 200 kg of grape collected at optimum technological maturity from two adjacent rows and placed in plastic bins and transported to the winery. The organic vinification protocol proposed by the Italian Association for Organic Farming (AIAB, Italy) was used; briefly, grapes were destemmed and crushed on the day of harvest and the grape must was placed in 200 l stainless-steel tanks and treated with sulfur dioxide (as potassium metabisulphite: 10 g/hl, AEB, Italy), complex nutrients (30 g/hl, Nutristart, Lafford, France) and inoculated with appropriate yeasts (20 g/hl *Saccharomyces cerevisiae*, F15, Laffort, France). Sugar consumption and temperature were monitored over time by means of a Babo densimeter throughout fermentation and the tank content was homogenised every day to dissolve the cap into the wine. Once fermentation was completed, the wine was transferred into smaller stainless steel tanks (100 l and 50 l size) for spontaneous clarification and malolactic fermentation. After the final racking, carried out 3 months from the end of alcoholic fermentation, the wines were cold-stabilized, then bottled and stored at 10 °C prior to chemical and sensory analyses.

2.3. Chemical analyses

Wines were analysed for the following parameters: alcohol strength (ALC, %), dry matter (DM, g/l), pH, total acidity (TA, g/l), volatile acidity (VA, g/l), optical density (AU) at 420, 520 and 620 nm, total colour intensity (CI, 420 + 520 + 620 nm AU), tonality (HUE, 420/520 nm AU) and total polyphenols at 280 nm (TP) according to European official methods (EC, 1990). Moreover, total and free sulfur dioxide (SO_2T and SO_2F, mg/l) (Ripper & Schmitt, 1896), reducing sugars (RS, g/l) (Lane & Eynon, 1923), anthocyanins (mg/l) (Arfelli, Chiavari, Castellari, & Amati, 1992), phenolic compounds (mg/l) (Castellari, Sartini, Fabiani, Arfelli, & Amati, 2002), ochratoxin A (mg/l) (Castellari, Fabbri, Fabiani, Amati, & Galassi, 2000), and biogenic amines (mg/l) (Moret & Conte, 1996) were also quantified by high performance liquid chromatography (HPLC) using a Dionex IC-500 system with diode array detection (Milano, Italy). More insights of the colour and phenolic components were obtained with the analysis of total colour (TC, AU), total polymeric pigments (TPP, expressed as % of total colour), co-pigmentation (Copig, expressed as % of total colour) and anthocyanins (ANT, expressed as % of total colour) (Boulton, 2001), large polymeric pigments (LPP, % of TPP), small polymeric pigments (SPP, % of TPP), tannins (TN, mg/l) and non-tannin total iron-reactive phenolics (IRP, mg/l) (Harbertson, Picciotto, & Adams, 2003) carried out by spectrophotometric assay (UV–Vis 1240 mini, Shimadzu, Milano, Italy). All the listed analyses were carried out at the end of malolactic fermentation; moreover, in order to monitor the change of wine composition over time, the analyses of colour and phenolic components were repeated 16 months from the end of the fermentation. Data are presented as mean values obtained from two replicated analyses of each duplicate vinification.

2.4. Volatile compound analyses

For wines produced in 2009, 20 ml samples were treated with 100 μl of internal standard solution (2-octanol: 500 mg/l in ethanol) before liquid–liquid extraction (Gerbi, Zeppa, & Carnacini, 1992) and the volatile compounds were analysed by injecting 1 μl of sample onto an ultra gas chromatograph interfaced with a DSQ single quadrupole mass spectrometer detector (Thermo Finnigan Trace GC, San Jose, CA) and equipped with a fused silica capillary column Stabilwax-DA (30 m, 0.25 mm i.d., 0.25 μm film thickness) (Restek, Bellefonte, PA). Chromatographic conditions were as follows: GC grade helium as the carrier gas (flow: 1.0 ml/min); splitless injection; detector temperature: 250 °C. The following temperature gradient programme was used: 45 °C, heated at 3 °C/min to 100 °C and then heated at 5 °C/min to 240 °C (held for 10 min). The MS-parameters were: detection by positive ion electron impact (EI) mass spectrometry, using an ionisation energy of 70 eV; transfer line at 280 °C; the global run time (45 min) was recorded in full scan mode (30–400 m/z mass range), with 1 scan being performed each second. Recognition of analyte was achieved by comparison of mass spectra with those of standards and/or those of NIST 2.0 (US National Institute of Standards and Technology) and Wiley 7 libraries. Linear retention index was calculated

for each compound and quantification (expressed as μg/l) was carried out, and considering the total ion current peak area in relation to the amount of the internal standard (IS), corrected by the response factor of each reference standard compound to 2-octanol. When a reference standard was not available, the correction factor (CF) was calculated by means of a compound with similar chemical structure.

For wine produced in 2010, the qualitative and quantitative volatile compounds were analysed by means of a headspace solid phase microextraction (SPME) procedure, with the same GC–MS instrument above described. A 10 ml aliquot of wine was transferred into a 20 ml glass vial containing 2 g of NaCl; then each vial was treated with 50 μl of 2-octanol internal standard solution (500 mg/l in ethanol) and the samples were carefully shaken to completely dissolve NaCl and left to equilibrate at room temperature before pre-incubation at 40 °C and extraction of volatiles with a polydimethylsiloxane (PDMS) SPME fibre (100 μm thickness) (Canuti et al., 2009). Chromatographic conditions, recognition and quantification of peaks were according to the methodology reported for wines of 2009, except for the oven temperature that was set according to the literature (Pons, Lavigne, Eric, Darriet, & Dubourdieu, 2008).

2.5. Electronic nose

A wine sample (10 ml) was poured into a glass vial and held at room temperature for 60 min for equilibration, then the headspace was analysed with the commercial portable electronic nose PEN2 (Airsense Analytics, Milano, Italy), composed of an array of ten temperature-moderated metal-oxide sensors (MOS), a sampling system, a data acquisition device and a data processing system, with the signal output of the sensors being digitized by recording and normalised to a value of 1.0 prior of sampling; this arbitrary baseline value was subtracted from the sensor responses prior to enhancement determination. A thermal desorption system was used to avoid sensor saturation due to ethanol. The signal output was measured at 1 s intervals for 100 s, long enough for the sensors to reach a steady state. The sensor values at 90 s were used for the pattern recognition studies.

2.6. Sensory evaluation

As a preliminary screening, the wines were tasted by enologists (2009's wines: 4 females and 6 males, aged between 25 and 63; 2010's wines: 5 females and 5 males, aged between 26 and 50) to ascertain differences between replicated vinifications. With this aim, two triangle tests (ISO, 2004) were set up (ORG_1 vs ORG_2, and BDN_1 vs BDN_2) with the enologists requested to identify the odd sample.

Afterwards, for each vintage one wine obtained from each viticultural management (ORG and BDN) was analysed for sensory differences in terms of "colour", "olfaction" and" taste" by a panel of judges (2009's wines: 8 females and 17 males, aged between 21 and 60; 2010's wines: 10 females and 15 males, aged between 21 and 55) recruited among employers and students of the Campus of Food Science (Cesena, Italy) and trained for paired comparison tests (ISO, 2005). Finally, a preference test was set up with consumers (2009's wines: 24 females and 40 males, aged between 20 and 68; 2010's wines: 26 females and 39 males, aged between 2 and 65) asked to indicate the preferred sample by means of a paired comparison test (ISO, 2005). Assessors were presented with transparent glasses (ISO, 1997) containing 30 ml of wine and asked to taste wines from left to right. Samples were coded with three-digit numbers and distributed in a completely randomized order.

2.7. Statistical analyses

Mean differences in chemical composition of wines were ascertained by one-way and two-ways analyses of variance (ANOVA), with interaction effect, using Fisher's LSD as *post hoc* test, and XLSTAT version 2011.1.05 (Addinsoft, Anglesey, UK). Principal components analysis (PCA) was used as an unsupervised multivariate data tool to find hidden structure among the observations (STATISTICA 8, Stat Soft, Tulsa, USA). For sensory analysis the results were evaluated using statistical tables of binomial distribution (Lawless & Heymann, 1998). All statistics were performed with significance at $p \leqslant 0.05$.

3. Results and discussion

3.1. Chemical analyses

The chemical analysis of the experimental wines (Table 1) indicated a satisfactory degree of grape ripeness and was consistent with the typical composition of the Sangiovese wine (Parenti, Spugnoli, Calamai, Ferrari, & Gori, 2004).

In particular, in 2009, the biodynamic preparations showed a significant reduction in terms of alcohol strength (ORG/2009: 13.4%; BDN/2009: 11.8%), volatile acidity (ORG/2009: 0.59 g/l; BDN/2009: 0.54 g/l), OD 420 (ORG/2009: 2.85 AU; BDN/2009: 2.21 AU), OD 520 (ORG/2009: 5.0 AU; BDN/2009: 3.31 AU), OD 620 (ORG/2009: 0.75 AU; BDN/2009: 0.55 AU), colour intensity (ORG/2009: 8.6 AU; BDN/2009: 6.1 AU), total polyphenols (ORG/2009: 1473 mg/l; BDN/2009: 1212 mg/l) and an increase of lactic acid (ORG/2009: 1.1 g/l; BDN/2009: 1.7 g/l) of wines at bottling (Table 1). However, in 2010, after 2 years of conversion, these differences were not significant, with the exception of OD 420 (ORG/2009: 2.10 AU; BDN/2009: 1.66 AU), total polyphenols (ORG/2010: 1157 mg/l; BDN/2010: 961 mg/l) and lactic acid (ORG/2009: 1.1 g/l; BDN/2009: 1.8 g/l). The malic acid was not detectable in any wines, due to its complete conversion into lactic acid during malolactic fermentation.

The two-way ANOVA disclosed a significant year effect for most of the analysed parameters (Table 1). The alcohol strength, volatile acidity, dry matter, reducing sugar, OD 420, OD 520, OD 620, colour intensity and total polyphenols were higher in 2009, whereas total acidity, pH and total SO_2 were higher in 2010 wines. This was most likely due to different average yields per plant (2009: 4.5 kg; 2010: 6.0 kg) as no shoot or bunch thinning was performed during the trial. It is well known that climatic conditions vary according to growing season, which is expected to affect key phenological stages, especially berry development. In fact, in 2009, a total rainfall of 190 mm was recorded from budburst to harvest whereas, for the same phenological stage in 2010, 455 mm of rainfall was recorded. Moreover, during berry maturation (i.e. from veraison to harvest) in 2009, negligible rainfall (19 mm) occurred and the average temperature was 24.5 °C, whereas 2010 was characterised by high rainfall (123 mm) and the average temperature was 21.3 °C. So, climatic conditions with increased water availability, are expected to reduce grape and wine colour and content of anthocyanins (Jackson & Lombard, 1993).

With regard to the management (ORG vs BDN), the ORG dominated over BDN, as evidenced by an 11% increase in alcohol strength (ORG = 12.7%; BDN = 11.4%), +15% in dry matter (ORG = 24.7 g/l; BDN = 21.5 g/l), 28% increase in OD 420 (ORG: 2.48 AU; BDN: 1.94 AU), 47% upper value in OD 520 (ORG: 3.95 AU; BDN: 2.69 AU), the +30% in OD 620 (ORG: 0.68 AU; BDN: 0.52 AU) +36% in colour intensity (ORG = 7.1 AU; BDN = 5.2 AU), +21% in total polyphenols (ORG = 1315 mg/l; BDN = 1087 mg/l), and +10% in total SO_2 (ORG = 34 mg/l; BDN = 31 mg/l) (Table 1). These results suggest that during the period of conversion the biodynamic management significantly reduced the basic wine

Table 1
One-way and two-ways ANOVA showing mean separation of basic chemical composition of Sangiovese red wines obtained from grapes managed with organic (ORG) or biodynamic (BDN) system during the 2009 and the 2010 season and the interactive effect of both factors.

ANOVA factor	Year of harvest/Mng	ALC (%)	TA (g/l)	VA (g/l)	pH	DM (g/l)	RS (g/l)	SO_2T (mg/l)	SO_2F (mg/l)	OD 420 (AU)	OD 520 (AU)	OD 620 (AU)	CI (AU)	HUE (AU)	TP (mg/l)	LA (g/l)
ORG	2009	13.4a	5.2b	0.59a	3.4a	26.5a	1.5a	30c	15a	2.85a	5.00a	0.75a	8.6a	0.57b	1473a	1.1b
	2010	12.0b	6.0a	0.40d	3.5a	23.0ab	1.2ab	38a	16a	2.10b	2.90bc	0.60ab	5.6bc	0.73ab	1157b	1.1b
BDN	2009	11.8b	5.2b	0.54b	3.4a	22.8ab	1.4ab	27d	13a	2.21b	3.31b	0.55b	6.1b	0.67ab	1212b	1.7a
	2010	11.1b	5.4b	0.50c	3.6a	20.3b	<1b	35b	15a	1.66c	2.06c	0.49b	4.2c	0.81a	961c	1.8a
Management (M)	ORG	12.7a	5.6a	0.48b	3.5a	24.7a	1.3a	34a	15a	2.48a	3.95a	0.68a	7.1a	0.65a	1315a	1.1b
	BDN	11.4b	5.3a	0.51a	3.5a	21.5b	1.2a	31b	14a	1.94b	2.69b	0.52b	5.2b	0.74a	1087b	1.7a
Year (Y)	2009	12.6a	5.2b	0.56a	3.4b	24.6a	1.4a	28b	14a	2.53a	4.16a	0.65a	7.3a	0.62a	1343a	1.4a
	2010	11.5b	5.7a	0.43b	3.5a	21.6b	1.1b	36a	15a	1.88b	2.48b	0.55b	4.9b	0.77a	1059b	1.4a
M × Y	*p*-value	0.219	**0.089**	**0.002**	0.822	0.717	0.612	0.360	0.615	0.394	0.199	0.321	0.227	0.917	0.536	0.742

Legend: Mng: management; ORG: organic management; BDN biodynamic management; ALC: alcohol strength; TA: titratable acidity; VA: volatile acidity; DM: total dry matter; RS: reducing sugars; SO_2T: total sulphur dioxide; SO_2F: free sulphure dioxide; CI: colour intensity; HUE: colour hue; TP: total polyphenols; MA: malic acid; LA: lactic acid; nd: not detectable. Unless specified data are the mean values of two independent vinifications of 200 kg (replicates). The letters represent the results of Fisher's LSD comparison tests: different letters on the column indicate means significantly different ($\alpha = 0.05$ or 0.1) among different wines. M × Y: interaction effect management × year. Significant values ($p < 0.05$) are shown in bold.

Table 2
One-way and two-ways ANOVA showing mean separation of anthocyanins and phenolics composition (mg/l) of Sangiovese red wines obtained from grapes managed with organic (ORG) or biodynamic (BDN) system during the 2009 and the 2010 season and the interactive effect of both factors.

ANOVA factor	Year of harvest/Mng	Anthocyanins					Phenolic acids		Cinnamic acids		Flavanols		Flavonols		Hydroxy stilbenes
		Df-3-glc	Cn-3-glc	Pt-3-glc	Pn-3-glc	Mv-3-glc	Prot	Gallic	Cout	Caft	(+)-Cat	(−)-Epicat	Rut	Quer	*t*-Resver
ORG	2009	8.9a	5.2a	12.2a	7.3a	40.9a	1.2a	17.0a	5.2c	13.0c	15.5a	9.5a	5.1b	nd	0.7a
	2010	4.7b	1.8c	7.5bc	3.6b	33.4a	nd	15.0a	11.9a	25.6a	4.8c	1.6b	10.3a	8.0a	nd
BDN	2009	5.3b	3.1b	7.8b	4.7b	35.2a	1.0b	17.8a	3.6c	9.7c	11.3b	9.5a	2.3b	nd	0.6b
	2010	2.6c	1.3c	4.2c	2.0c	22.6a	nd	14.7a	8.8b	20.0b	6.3c	2.3b	5.3b	4.6b	nd
Management (M)	ORG	6.8a	3.5a	9.8a	5.4a	37.1a	0.6a	16.0a	8.6a	19.3a	10.1a	5.8a	7.7a	3.9a	0.3a
	BDN	3.9b	2.2b	5.9b	3.3b	28.9a	0.5b	16.3a	6.2b	14.9b	8.8a	5.6a	3.8b	2.3b	0.2b
Year (Y)	2009	7.1a	4.2a	10.0a	6.0a	38.0a	1.1a	17.4a	4.4b	11.4b	13.4a	9.5a	3.7b	ndb	0.6a
	2010	3.6b	1.5b	5.8b	2.8b	28.0a	ndb	14.9b	10.4a	22.8a	5.5b	1.9b	7.8a	6.3a	ndb
M × Y	*p*-value	0.151	**0.042**	0.601	0.219	0.616	**0.040**	0.583	0.308	0.458	**0.014**	0.332	0.367	**0.001**	**0.001**

Legend: Mng: management; Df: delphinidin; Cn: cyanidin; Pt: petunidin; Pn: peonidin; Mv: malvidin; glc: glucoside; Prot: protocatechuic acid; Cout: coutaric acid; Caft: caftaric acid; Cat: catechin; Epicat: epicatechin; Rut: rutin; Quer: quercetin; *t*-resver: *trans*-resveratrol; nd: not detected, i.e. below the limit of quantification; The letters represent the results of Fisher's LSD comparison tests: different letters on the column indicate means significantly different ($\alpha = 0.05$) among different wines. M × Y: interaction effect management × year. Significant values ($p < 0.05$) are shown in bold.

characteristics, such as chemical components and colour components, possibly due to influence on berry composition (Picone, Laghi, Olivi, Rombolà, & Capozzi, 2013). First-order interactive effects between management (M) and year (Y) were limited to volatile acidity (α: 0.05).

3.2. Phenolic compounds

Due to the importance of polyphenolic compounds of red wine as natural dietary antioxidants that affect the quality of red wines in terms of astringency, bitterness and colour, further analyses of monomeric anthocyanins, simple phenols and phenolic acids were carried out by HPLC (Table 2). As for basic chemical parameters, after the first year of treatment with biodynamic *preparations* (2009) the contents of phenolic compounds significantly decreased in BDN wines, e.g. delphinidin-3-glucoside (ORG: 8.9 mg/l; BDN: 5.3 mg/l), cyanidin-3-glucoside (ORG: 5.2 mg/l; BDN: 3.1 mg/l), petunidin-3-glucoside (ORG: 12.2 mg/l; BDN: 7.8 mg/l), peonidin-3-glucoside (ORG: 7.3 mg/l; BDN: 4.7 mg/l), as well as (+)-catechin (15.5 vs 11.3 mg/l), protocatechuic acid (ORG: 1.2 mg/l; BDN: 1.0 mg/l) and *trans*-resveratrol (ORG: 0.7 mg/l; BDN: 0.6 mg/l). In 2010, after 2 years of field treatment with biodynamic *preparations*, a few significant differences were still present, such as delphinidin-3-glucoside (ORG: 4.7 mg/l; BDN: 2.6 mg/l) and peonidin-3-glucoside (ORG: 3.6 mg/l; BDN: 2.0 mg/l), although, for these compounds, the difference between ORG and BDN wines in 2010 was minimum compared to the previous vintage. This trend is consistent with recent findings that have demonstrated a lack of significant difference in total anthocyanins wines measured by spectrophotometry as variation of absorbance units per litre (ΔAbs/l) between organic and biodynamic Lambrusco red, whereas polyphenol concentration peaked in biodynamic wine from a settled/long run management (Tassoni, Tango, & Ferri, 2014). Our study provided a further insight into phenolics composition of red wines, and the 2010 harvest showed significant differences in coutaric (ORG: 11.9 mg/l; BDN: 8.8 mg/l), caftaric acids (ORG: 25.6 mg/l; BDN: 20.0 mg/l) and flavonols (rutin: 10.3 vs 5.3 mg/l; quercetin: 8.0 vs 4.6 mg/l) as well (Table 2).

The concentration of flavonoids and non-flavonoids under investigation varied according to the management and year of harvest (Table 2). In fact, the two-way ANOVA showed a significant enhancing effect of ORG management on all these parameters with the exception of malvidin-3-glucoside, gallic acid, (+)-catechin and

(−)-epicatechin, and this was in agreement with previous measurements of colour parameters and total polyphenols, and higher value of delphinidin-3-glucoside (ORG: 6.8 mg/l; BDN: 3.9 mg/l), cyanidin-3-glucoside (ORG: 3.5 mg/l; BDN: 2.2 mg/l), petunidin-3-glucoside (ORG: 9.8 mg/l; BDN: 5.9 mg/l) and peonidin-3-glucoside (ORG: 5.4 mg/l; BDN: 3.3 mg/l), protocatechuic (ORG: 0.6 mg/l; BDN: 0.5 mg/l), coutaric (ORG: 8.6 mg/l; BDN: 6.2 mg/l), caftaric (ORG: 19.3 mg/l; BND: 14.9 mg/l), rutin (ORG: 7.7 mg/l; BDN: 3.8 mg/l), *trans*-resveratrol (ORG: 0.3 mg/l; BDN: 0.2 mg/l) and quercetin (ORG: 3.9 mg/l; BDN: 2.3 mg/l) were detected in ORG wines. The concentrations of caffeic and coumaric acids, as well as myricetin, were below the detection limit (data not shown).

With regard to harvest seasons, the 2009 vintage showed differences in delphinidin-3-glucoside (ORG: 7.1 mg/l; BDN: 3.6 mg/l), cyanidin-3-glucoside (ORG: 4.2 mg/l; BDN: 1.5 mg/l), petunidin-3-glucoside (ORG: 10.0 mg/l; BDN: 5.8 mg/l) and peonidin-3-glucoside (ORG: 6.0 mg/l; BDN: 2.8 mg/l) as well as protocatechuic acid (ORG: 1.1 mg/l; BDN: nd) and gallic acid (ORG: 17.4 mg/l; BDN: 14.9 mg/l), (+)-catechin (ORG: 13.4 mg/l; BDN: 5.5 mg/l), (−)-epicatechin (ORG: 9.5 mg/l; BDN: 1.9 mg/l) and *trans*-resveratrol (ORG: 0.6 mg/l; BDN: nd) (Table 2). Conversely, in 2010, higher concentration of cinnamic acids, such as coutaric (BDN: 10.4 mg/l; ORG: 4.4 mg/l) and caftaric (BDN: 22.8 mg/l; ORG: 11.4 mg/l) and flavonols, such as rutin (BDN: 7.8 mg/l; ORG: 3.7 mg/l) and quercetin (BDN: 6.3 mg/l; ORG: nd) were present.

Significant vineyard management × year interaction occurred for cyanidin-3-glucoside, protocatechuic acid, (+)-catechin, quercetin and *trans*-resveratrol, demonstrating a strong relationship between these two factors and some flavonoids and not-flavonoid compounds (Table 2).

3.3. Tannins and colour components

The variations in anthocyanins (ANT) and tannins (TN) and their impact on the formation of small (SPP), large (LPP), and total polymeric pigments (TPP = SPP + LPP), as well as the content of total iron-reactive phenols (IRP) and chromatic characteristics, such as total colour (TC) and co-pigmentation (CP), are listed in Table 3. In order to obtain more insight about the evolution of these compounds over time, the analyses were performed at bottling (ORG/2009, ORG/2010, BDN/2009, BDN/2010) and 1 year after storage ($ORG/2009_{(2011)}$, $ORG/2010_{(2012)}$, $BDN/2009_{(2011)}$, $BDN/2010_{(2012)}$) in each management practice.

Yearly data analysis, in each management practice, showed that the total colour (TC) at bottling was significantly higher in ORG wines produced in 2009 (4.1 AU), which supported the colour intensity data of basic analyses. In these wines, higher values were also obtained for tannins (1191 mg/l) and total phenols (1856 mg/l). The co-pigmentation and anthocyanins were comparable between ORG and BDN wines obtained in the same harvest and analysed at bottling whereas large and small polymeric pigments were comparable in 2010's wines. Inspection of the results of a one-way ANOVA performed on each field management (Table 3) revealed a significant decline in BDN wines for total colour (ORG: 3.0 AU; BDN: 2.2 AU), tannins (ORG: 926 mg/l; BDN: 630 mg/l) and total polyphenols (ORG: 1532 mg/l; BDN: 1150 mg/l). However, in BDN wines, a higher value of TPP, especially in LPP, was recorded. With regard to the season, the 2009 recorded higher values for total colour, co-pigmentation, anthocyanins, tannins and total phenols whereas, in 2010, an increase in total polymeric pigment, including LPP and SPP, occurred. One year of storage significantly modified the content of these compounds in wines produced in 2009, resulting in a significant reduction in total colour (2009: 3.5 AU; $2009_{(2011)}$: 2.5 AU) co-pigmentation (2009: 13.5%; $2009_{(2011)}$: 0.95%), anthocyanins (2009: 49.8%; $2009_{(2011)}$: 26.8%), tannins (2009: 1005 mg/l; $2009_{(2011)}$: 797 mg/l) and total polyphenols (2009: 1634 mg/l; $2009_{(2011)}$: 1423 mg/l). Spectrophotometric analysis after 1 year showed that, for the wines produced in 2009, the polymerisation of pigments was higher compared to wine produced in 2010, but with decreasing monomeric pigments and co-pigmentation. It seems that a high concentration of anthocyanin was initially favourable for increasing the formation of polymeric pigments, and the concurrent loss of free anthocyanins is enhanced over time. The decrease in co-pigmentation was expected during wine storage, especially during the first year of wine aging as cofactors are oxidised or hydrolysed (Boulton, 2001). The loss of tannins and anthocyanins during storage may be explained by anthocyanin degradation or incorporation of these compounds into oligomeric and polymeric pigments with a general preferential

Table 3
Statistical analysis (one-way and two-ways ANOVA) of the phenolic and colour components of ORG and BDN Sangiovese red wines. Data represent the mean value of two vinifications.

ANOVA factor	Year/Mng	TC (AU)	TPP (%)	Copig (%)	ANT (%)	LPP (%)	SPP (%)	TN (mg/l)	IRP (mg/l)
ORG	2009	4.1a	34.4g	15.4a	50.2a	18.2e	16.3d	1191a	1856a
	2010	2.6bc	51.7e	7.9bc	40.5b	32.9cd	18.8cd	700cd	1248cd
BDN	2009	2.9b	38.9f	11.6ab	49.5a	27.4d	11.5e	818bcd	1413bc
	2010	1.9d	55.6d	3.6cd	40.8b	37.3c	18.3cd	465e	936e
ORG	$2009_{(2011)}$	3.0b	70.7b	0.4d	28.9cd	44.6ab	26.1a	960b	1623ab
	$2010_{(2012)}$	2.6bcd	55.2d	10.2ab	34.5bc	34.3cd	20.9bc	854bc	1403bc
BDN	$2009_{(2011)}$	2.1cd	73.7a	1.5d	24.8d	49.0a	24.7ab	635cde	1224cd
	$2010_{(2012)}$	1.8d	59.0c	10.7ab	30.3cd	38.7bc	20.3bcd	600de	1026de
Management (M)	ORG	3.0a	53.0b	8.5a	38.5a	32.5b	20.5a	926a	1532a
	BDN	2.2b	56.8a	6.9a	36.3a	38.1a	18.7a	630b	1150b
Year (Y)	2009	3.5a	36.7d	13.5a	49.8a	22.8c	13.9c	1005a	1634a
	2010	2.2b	53.6c	5.7b	40.6b	35.1b	18.5b	582c	1092c
	$2009_{(2011)}$	2.5b	72.2a	0.95c	26.8c	46.8a	25.4a	797b	1423b
	$2010_{(2012)}$	2.2b	57.1b	10.5a	32.4c	36.5b	20.6b	626bc	1114c
M × Y	*p*-value	0.769	0.863	0.427	0.727	0.613	0.453	0.739	0.870

Legend: Mng: management; when not specified between parentheses (year) analyses are meant at bottling (4 months after the end of fermentation); In $ORG/2009_{(2011)}$, $BDN/2009_{(2011)}$, $ORG/2010_{(2012)}$ and $BDN/2010_{(2012)}$, analyses are performed at 12 months bottling (16 months after the end of fermentation). TC: total colour; TPP: total polymeric pigments; Copig: copigmentation; ANT: anthocyanins; LPP: large polymeric pigments; SPP: small polymeric pigments; TN: tannins; IRP: iron-reactive phenolics. The letters represent the results of Fisher's LSD comparison *post hoc* test: different letters on the column indicate mean significantly different ($\alpha = 0.05$) among different wines. M × Y: interaction effect of management × year. Significant difference for $p < 0.05$.

formation of pigmented tannin-anthocyanin polymers (LPP) over anthocyanin-acetaldehyde cross-linked oligomers and pyranoanthocyanins (SPP) during aging (Harbertson et al., 2003). This hypothesis was reinforced by the results obtained on polymeric pigments. In fact, after 1 year of storage, the total polymeric pigments, fractionated into LPP and SPP, increased significantly (plus 101% and 80%, respectively). To some degree, a rearrangement of phenolic compounds was also observed in 2010 wines after 1 year of storage. The increase in single LPP and SPP fractions showed a significant enhancement in the total polymeric pigments content (+6%). The less pronounced formation of LPP and SPP in the wines of the 2010 season than in the wines of the 2009 season during 1 year storage might be due to low levels of anthocyanins and tannins in 2010, i.e. the molar ratio between these compound is altered. The anthocyanins decreased (2010: 40.6%; $2010_{(2012)}$: 32.4%), whereas total colour, tannins and total polyphenols did not differ significantly.

Further conclusions about tannin evolution are premature due to fact that the polymerisation of flavanols, with or without anthocyanins, is a slower phenomenon than the reactivity or degradation of anthocyanins (Arapitsas, Speri, Angeli, & Mattivi, 2014). No significant differences were found in any chemical parameters when the interaction between management and year was analysed (Table 3).

3.4. Volatile compound analyses

The electronic nose was used as a preliminary screening approach to disclose clustering among samples, using the PCA that explained 95% of total variance with the first two components, accounting for 73.8% and 18.6%, respectively. The signals generated by the sensors (and merged in the PCA plot) showed that BDN/2009 and BDN/2010 were clustered in the upper-right side, well separated from the ORG/2009 and ORG/2010 (data not shown).

Following this observation, the wines were analysed for their volatile composition by means of GC–MS. The volatiles' pattern profiles of ORG and BDN wines produced in 2009 were similar (Table 4). However, from ~~a~~ quantitative perspective, the ORG/2009 wines were highest in alcohols such as *t*-3-hexen-1-ol (2302 vs 8 μg/l, note of grass) and 2,3-butanediol (9157 vs 5789 μg/l, microbial origin from acetoin, butter), esters (ethyl 4-hydroxybutyrate 12788 vs 8786 μg/l, caramel), acids (acetic acid, 18186 vs 11930 μg/l, vinegar) and other compounds, such as *t*-5-hydroxy-2-methyl-1,3-dioxane (175 vs 129 μg/l, cooked banana leaf, floral, honey, rose honey). Conversely, BDN/2009 wines were most abundant in *c*-3-hexen-1-ol (16 vs 8 μg/l, green) and 1H-Indole-3-ethanol (7481 vs 2719 μg/l, almond).

Similar results were obtained for the 2010 vintage in which the ORG wines were characterised by higher concentrations of volatiles compared to the BDN wines (Table 5) in terms of esters (note of ethyl butyrate, apple, fruity), e.g. ethyl octanoate (811% vs 648%, note of apricot, fruity), ethyl decanoate (713% vs 412%, note of grape, brandy), isopentyl octanoate (14.2% vs 10.3%, note of fruity), ethyl dodecanoate (76.1% vs 53.5%, note of floral), isoamyl decanoate (5.9% vs 3.4%, note of waxy, banana, fruity, sweet, cognac, green). By contrast BDN wines were characterised by highest concentrations of 3-nonalol (0.5% vs nd, note of spice, herbal), esters such as hexyl acetate (1.7% vs 0.45%, note of fruity, apple, cherry, pear, floral), ethyl lactate (2.3% vs 1.5%, note of fruity, buttery), diethyl succinate (33.4% vs 8.3%, note of mild fruity, cooked apple) and 1-methyl-4-(1-methylethylidene)-cyclohexene (1.3% vs nd, note of fresh woody sweet pine citrus). It is noteworthy that the few significant differences recorded between ORG and BDN wines in 2009 became less evident in 2010.

Table 4
Volatiles composition of ORG and BDN Sangiovese red wines (μg/l) from 2009 harvest (mean value of two vinifications).

Compound (μg/l)	Wine	
	ORG	BDN
Alcohols		
Isobutyl alcohol	37414	35873
n-Butanol	404	293
Isoamyl alcohol	96789	95056
2-Hexanol	52	52
4-Methyl-1-pentanol	19	19
3-Methyl-1-pentanol	68	53
3-Pentanol	12	5
1-Hexanol	711	765
t-3-Hexen-1-ol	**2302a**	**8b**
c-3-Hexen-1-ol	**8b**	**16a**
3-Ethoxy-1-propanol	22	36
2-Ethyl-1-hexanol	13	25
2,3-Butanediol	**9157a**	**5789b**
n-Dodecan-1-ol	59	65
Esters		
Isoamyl acetate	709	493
Ethyl caproate	161	149
Ethyl lactate	38827	46238
Ethyl caprylate	278	224
Ethyl 3-hydroxy butyrate	583	463
Ethyl decanoate	7	5
Diethyl succinate	1042	1724
Methyl-4-hydroxybutyrate	124	77
Ethyl 4-hydroxybutyrate	**12788a**	**8786b**
β-Phenylethyl acetate	95	45
N-acetylglycine ethyl ester	243	260
Butanedioic acid, monoethyl ester	222718	235353
Acids		
Acetic acid	**18186a**	**11930b**
Propanoic acid	1528	1596
Isobutyric acid	1529	3346
n-Butyric acid	531	471
Pentanoic acid + dioxane	2916	2681
Hexanoic acid	1337	1369
Octanoic acid	1548	1543
Nonanoic acid	460	441
Decanoic acid	266	229
Dodecanoic acid	84	80
Hexadecanoic acid	196	137
Miscellaneous		
3-(Methylthio) propanol	1823	2494
β-Phenylethyl alcohol	46597	46168
3-Hydroxy-2-butanone	5163	1488
t-5-Hydroxy-2-methyl-1,3-dioxane	**175a**	**129b**
t-4-Hydroxy-2-methyl-1,3-dioxane	180	129
γ-Butyrolactone	13253	11689
4-Hydroxy-2-butanone	956	1003
c-5-Hydroxy-2-methyl-1,3-dioxane	27	20
N-(3-Methylbutyl) acetamide	432	586
1,4-Diacetoxybutane + benzyl alcohol	241	368
2H-Pyran-2,6(3H) dione	319	268
Benzaldehyde 4-pentyl	425	440
Dihydro-5-(1-hydroxyethyl)-2-(3H) furanone	816	1142
1H-Indole-3-ethanol	**2719b**	**7481a**
4-Hydroxy-benzeneethanol	29347	24125

The letters represent the results of Fisher's LSD comparison *post hoc* test: bold and different letters on the column indicate significant difference ($\alpha = 0.05$) among wines.

3.5. Sensory evaluation

Preliminary investigation, performed by enologists, established consistency among replicated vinifications in both vintages. Thus, ORG and BDN wines were analysed by year of harvest at bottling, first for differences in terms of "colour", "aroma" and" taste" by a panel of judges trained for paired comparison tests, and then for preference by a panel of consumers.

Table 5
Volatiles composition of ORG and BDN Sangiovese red wines from 2010 harvest. Data are expressed as percentage of the internal standard GC area (ISA); (mean value of two vinifications).

Compound (peak area)	Wine	
	ORG	BDN
Alcohols		
Isobutyl alcohol	12.2	13.3
Isoamyl alcohol	268	231
1-Hexanol	4.0	4.1
3-Nonalol	***ndb***	**0.5a**
n-Dodecan-1-olo	nd	0.7
Esters		
Ethyl acetate	34	27
Ethyl isobutyrate	1.4	1.0
Ethyl butyrate	**3.5a**	**2.5b**
Ethyl 2-methylbutyrate	1.4	1.3
Ethyl isovalerate	2.3	1.8
Isoamyl acetate	40	48
Ethyl hexanoate	95	95
Hexyl acetate	**0.4b**	**1.7a**
Ethyl heptanoate	1.2	0.5
Ethyl 2-hexenoate	0.4	0.3
Ethyl lactate	1.5b	2.3a
Methyl octanoate	0.7	0.2
Ethyl octanoate	**811a**	**648b**
3-Methylbutyl octanoate	5.1	4.8
Ethyl nonanoate	2.7	2.2
Ethyl 2-hydroxy-4-methylvalerate	0.3	0.5
Isobutyl octanoate	0.7	1.6
Methyl decanoate	1.7	1.2
Ethyl decanoate	**713a**	**412b**
Isopentyl octanoate	**14.2a**	**10.3b**
Diethyl succinate	**8.3b**	**33.4a**
Ethyl 9-decenoate	44	31
β-Phenylethyl acetate	2.7	2.0
Ethyl dodecanoate	**76.1a**	**53.5b**
Isoamyl decanoate	**5.9a**	**3.4b**
Monoethyl succinate	26.4	20.1
Ethyl tetradecanoate	6.9	2.8
Acids		
Hexanoic acid	0.4	0.3
Octanoic acid	22.1	23.6
Nonanoic acid	0.1	0.4
Decanoic acid	16.3	17.3
Miscellaneous		
β-Phenethyl alcohol	17.6	38.9
n-Tetradecane	1.8	1.3
1-Methyl-4-(1-methylethylidene)-cyclohexene)	***ndb***	**1.3a**
Benzaldehyde	2.0	2.2
Naphthalene	0.7	3.8
β-Damascenone	1.2	1.4
(S)-1,2-Dihydro-4,7-dimethyl-1-isopropylnaphthalene	7.9	7.3
4-Ethylguaiacol	3.2	2.2
4-Ethylphenol	1.7	1.9
Naphthalene, 4-isopropyl-1,6-dimethyl	2.3	1.9

The letters represent the results of Fisher's LSD comparison *post hoc* test: bold and different letters on the column indicate significant difference ($\alpha = 0.05$) among wines; nd: not detected.

For both vintages, the trained judges observed differences only in colour whereas consumers showed no preference for any wine (data not shown).

3.6. Ochratoxin A and biogenic amines

In all wines, regardless of management and year, the concentration of OTA was below the detection limit (LOQ: 0.020 μg/l). A similar pattern was observed for biogenic amines with the concentrations of putrescine, cadaverine, tyramine, methylamine, agmatine, spermine and spermidine, whereas histamine was detected in one out of two replicates of ORG/2009 (0.22 mg/l). This concentration is not considered to have a detrimental effect on human health, taking into account the recommended limits of 2–10 mg/l set by several countries.

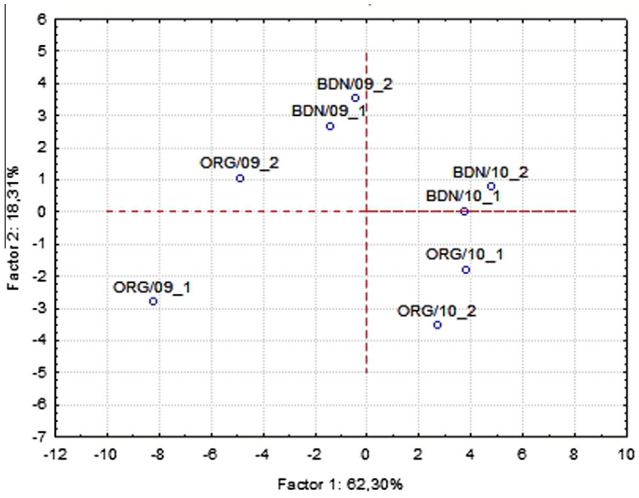

Fig. 1. Score plot of the ORG and BDN wines clustered according to significant chemical parameters, i.e. basic analyses, colour components, phenolic and volatile compounds.

3.7. Principal components analysis of wines

To give a final overview of this study, the parameters that showed significant differences between the two wines were analysed by means of PCA (Fig. 1) that explained 81% of the variability with the first two components ($F_1 = 62.3\%$; $F_2 = 18.3\%$). Both wines (ORG and BDN with replications) produced in 2010 were positioned in the positive quadrant of the PCA whereas the negative quadrant contained wines produced in 2009. Besides an evident "year" effect, it is noteworthy that wines made from either ORG or BDN management practices shared space in the experimental plot, with the exception of ORG/09_1. These results suggested that the biodynamic *preparations*, used over the 2 years of investigation, significantly influenced the chemical and sensory characteristics of wines.

4. Conclusions

This study provides significant evidence of the composition of sustainable wines made by biodynamic and organic management practices. The quality of Sangiovese red wines was affected, to a large extent, by the 'on-field' application of biodynamic '*preparations*'. The effect was independent of the season, although 2009 and 2010 were characterised by different climatic conditions. Sensory evaluation confirmed that the biodynamic preparations were influential in reducing colour intensity, whereas the consumers showed a lack of preference for either wine, regardless of the year of production. It is noteworthy that, in general, the differences between ORG and BDN wines diminished during the second year of vineyard management, probably due to changes induced by biodynamic preparations on berry composition, ensuing from modifications at plant and soil levels. The results of this study warrant further investigation to improve the understanding of the effect of biodynamic management on wine quality.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2014.06.093.

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