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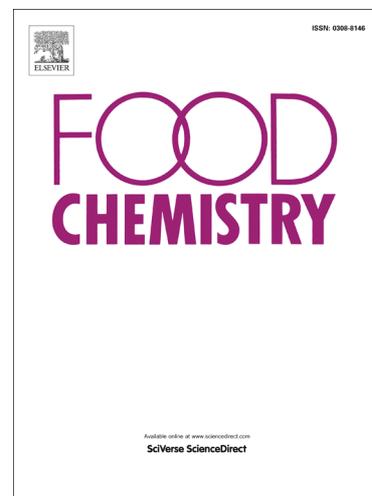
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Comparison of Sangiovese wines obtained from stabilized organic and biodynamic vineyard management systems

Running title: Characterization of sustainable wines

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ABSTRACT

Sangiovese red wines produced from organic (ORG) and biodynamic (BDN) vineyards over two consecutive vintages (2011 and 2012) were compared for chemical and sensory parameters to investigate a sustainable approach to grape production. The effects of management practice, vintage, and their interaction were investigated. The ORG wines showed higher total acidity and lower volatile acidity and pH. Although trained panelists highlighted some differences in astringency and odor complexity between ORG and BDN wines, consumers had no preference. The concentrations of anthocyanins, phenolic and cinnamic acids, and flavonols, as well as colour components, did not differ—contrary to results from the conversion period from ORG to BDN (2009 and 2010) in the same vineyard (Parpinello, Rombolà, Simoni, & Versari, 2015). Together, these two studies demonstrate that ORG and BDN wine characteristics tend to be similar after the first year of conversion, indicating that the BDN method can produce high-quality Sangiovese wine.

Keywords: biodynamic, organic, wine sustainability, bioactive compounds, volatiles compounds, sensory evaluation

1. Introduction

An environmentally sustainable approach to wine production has been garnering interest among producers and consumers. As a result, the effect of organic and biodynamic practices in the vineyard on wine quality and consumer perception has become an object of great interest by researchers (Castellini, Mauracher, & Troiano, 2017; Mann, Ferjani, & Reissig, 2012; Moneyron,

Lallemand, Schmitt, Perrin, Soustre-Gacougnolle, & Masson, 2017; Parpinello, Rombolà, Simoni, & Versari, 2015; Preston, 2008; Turinek, Grobelnik-Mlakar, Bavec, & Bavec, 2009; Zucca, Smith, & Mitry, 2009). The main difference between biodynamic and organic management is that the former uses specific fermented preparations proposed by Steiner (1861–1925), which are believed to stimulate the soil nutrient cycle, enforce photosynthesis, and optimize the evolution of compost enhancing in both soil and crop (Koepf, Schaumann, & Haccius, 2001). Several studies have already compared organic and conventional wines in terms of overall discrimination (Cozzolino, Holdstock, Damberg, Cynkar, & Smith, 2009), phenolic compounds and antioxidant activity (Miceli, Negro, Tommasi, & De Leo, 2003; Tintunen & Lehtonen, 2001), physico-chemical and sensory characteristics (Martin & Rasmussen, 2011; Mulero, Pardo, & Zafrilla, 2009; Mulero, Zafrilla, Cayuela, Martinez-Cacha, & Pardo, 2011), biogenic amines (Kalkan Yildirim, Üren, & Yücel, 2007; Yañez, Saavedra, Martínez, Córdova, & Ganga, 2012), and ochratoxin A (Miceli, Negro, Tommasi, & De Leo, 2003; Plahuta & Raspor, 2007; Ponsone, Combina, Dalcero, & Chulze, 2007).

However, there is a need for more information about the differences between organic and biodynamic vineyard management in order to better understand their effects on grape and wine composition. Previous research has shown that biodynamic Steiner's preparations enhanced the vegetative-reproductive balance of plants in the vineyard (Reeve, Carpenter-Boggs, Reganold, York, McGourty, & McCloskey, 2005). The preparations improved soil biomass (Zaller & Köpke, 2004) and the enzymatic activities associated with induced plant resistance, which are correlated with plant biotic and abiotic stresses (Botelho, Roberti, Tessarin, Garcia-Mina, & Rombolà, 2016). Many studies have also focused on the variations in soil-born microbial and earthworm communities as a result of changes in soil properties due to different agricultural management practices, such as conventional, organic, or biodynamic (Burns, Bokulich, Cantu, Greenhut, Kluepfel, O'Geen, et al., 2016; Faust, Heinze, Ngosong, Sradnick, Oltmanns, Raupp, et al., 2017; Gaigher & Samways, 2010; Zaller & Köpke, 2004). Recently, a study (Picone, Trimigno, Tessarin,

Donnini, Rombolà, & Capozzi, 2016) used ^1H NMR to demonstrate a diverse grape berry metabolome, possibly associated with the different physiological response of plants to either organic or biodynamic management. In particular, lower amounts of sugars and coumaric, and caffeic acids, as well as a higher amount of γ -aminobutyric acid (GABA) were observed in biodynamic grapes. Other authors (Doring, Frisch, Tittmann, Stoll, & Kauer, 2015) report that the growth and yield of grapevines under non-integrated management practices (organic and biodynamic) decreased compared to integrated management. However, fruit quality was not affected by the management system. With regard to aspects which could affect the vinification process, most research has used a genomic approach to address grape and wine microbial diversity, which increases under non-conventional management (Ghosh, Bagheri, Morgan, Divol, & Setati, 2015; Guzzon, Gugole, Zanzotti, Malacarne, Larcher, von Wallbrunn, et al., 2016; Mezzasalma, Sandionigi, Bruni, Bruno, Lovicu, Casiraghi, et al., 2017; Patrignani, Montanari, Serrazanetti, Braschi, Vernocchi, Tabanelli, et al., 2016). Additionally, the ^1H -NMR (Laghi, Versari, Marcolini, & Parpinello, 2014) and droplet evaporation methods (Kokornaczyk, Parpinello, Versari, Rombolà, & Betti, 2014) were able to discriminate between red wines from organic and biodynamic grapes. Other researchers focused their attention on a potential hazard effect (Plahuta & Raspur, 2007), sensory differences (Ross, Weller, Blue, & Reganold, 2009), or biogenic amines and phenolic compounds (Tassoni, Tango, & Ferri, 2013). A previous publication by the same authors (Parpinello, Rombolà, Simoni & Versari, 2015) reported the effects of biodynamic production practices on the composition (chemicals, phenolics, aromatics) and sensory attributes of Sangiovese red wines during 2009 and 2010, the two years the vineyard was being converted from organic to biodynamic. The results show that the wine quality during the first year of conversion was affected, to a large extent, by the on-field application of biodynamic preparations. In fact, the biodynamic wines were characterized by lower alcohol strength, colour intensity, and phenolics (total polyphenols, anthocyanins, catechin), whereas the GC/MS aroma profiles were comparable between wines. In the second year, the biodynamic wines still differed from the organic ones in

terms of phenolics (total polyphenols, polymeric pigments, co-pigmentation, tannins, and iron-reactive polyphenols), but the differences—although still significant—had diminished.

The present study aims at improving the understanding of the effect of sustainable vineyard management practices on the composition of Sangiovese red wines produced after the two-year conversion period (during which the ORG or BDN management effects are still stabilizing). The present data, together with those already published by the same authors (Study 1), were obtained from trials conducted over four years of ORG and BDN management (vintages 2009–2012) in the same vineyard in the Emilia-Romagna region of Italy using the main autochthonous red grape variety grown in Italy, Sangiovese. Thus, this study provides important information for evaluating the differences between BDN and ORG vineyard management.

2. Materials and methods

2.1. *Vineyard management*

Grapes (Sangiovese, clone FEDIT 30 ESAVE) were harvested for two seasons (2011 and 2012) from both organic (ORG) and biodynamic (BDN) vineyards. The vineyard, approximately 2 ha located in Tebano (Ravenna, Italy; 44°17'24.9"N; 11°47'08.6"E), had been managed as organic in accordance with Reg. EC 834/2007 (EC, 2007) since 2007, with neither water irrigation nor fertilization. The vineyard had vine by row spacing of 1 x 2.8 m, corresponding to 3571 spur-pruned vines per ha. In 2009 about 50% of the vineyard was converted to biodynamic management using Steiner's preparations as previously reported (Study 1) and, as previously reported (Botelho, Roberti, Tessarin, Garcia-Mina, & Rombolà, 2016), each organic or biodynamic area (2 ha) was divided into seven experimental parcels. After the period of conversion from ORG to BDN (2009 and 2010), some actions were carried out in order to control the vine/yield in 2011 and 2012. The

numbers of buds (12–14) and bunches (11–15) were adjusted by winter pruning and cluster thinning, respectively. These two actions were performed to improve microclimate conditions in the canopy, reduce vine susceptibility to pathogens, control the yield, and increase berry and wine quality. At the end of each vegetative season, herbaceous species, such as fava bean (*Vicia faba*), barley (*Hordeum vulgare*), subterranean clover (*Trifolium subterraneum*), and mustard green (*Brassica juncea*), were sown in alternate planting rows in both organic and biodynamic plots. The soil was managed by mowing the vegetation during late spring, which maintained biomass on the soil surface.

2.2. Winemaking protocol

The vinification protocol used during the trial met the prerequisites of the EU regulations for organic wine production (EC, 2012). In the 2011 and 2012 harvests, independent vinifications of 200 kg of grapes were set up in duplicate for both viticultural management methods (Organic: ORG/11 and ORG/12; Biodynamic: BDN/11 and BDN/12), for a total of eight wines. For each vinification, grapes were collected at optimum technological maturity in small bins (approximately 20 kg) from 50 vines per parcel in two adjacent rows and immediately transported to the winery. The vinification protocol proposed by the Italian Association for Organic Farming (AIAB, Italy) was adopted as follows: the same day of harvest the grapes were destemmed and crushed, and the must was placed in 200-litre stainless-steel tanks. Sulfur dioxide (as potassium metabisulphite: 10 g/hl, AEB, Italy) and complex nutrients (30 g/hl, Nutristart, Lafford, France) were added, and the must was inoculated with the selected yeast (20 g/hl *Saccharomyces cerevisiae*, F15, Laffort, France). Ongoing fermentation and temperature (°C) were monitored over time throughout sugar consumption by means of a Babo densimeter, and the must/pomace was homogenized daily by manual punching down. Once fermentation was completed, the wine was racked into smaller

stainless-steel tanks (100 l and 50 l) for spontaneous clarification and malolactic fermentation. Three months after the end of alcoholic fermentation a final racking was carried out. The wines were then cold-stabilized, bottled, and stored at 10°C until chemical and sensory analyses were conducted.

2.3. Chemical analyses

Samples were analyzed according to European official methods (EC, 1990) for the following parameters: alcohol strength (ALC, %); pH; total acidity (TA, g/l); volatile acidity (VA, g/l); dry matter (DM, 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). Moreover, total and free sulfur dioxide (SO₂T and SO₂F, mg/l), reducing sugars (RS, g/l), anthocyanins (mg/l) (Arfelli, Chiavari, Castellari, & Amati, 1992), and phenolic compounds (mg/l) (Castellari, Sartini, Fabiani, Arfelli, & Amati, 2002) were analyzed. The following colour and phenolic components were analyzed by spectrophotometric assay (UV-Vis 1240 mini, Shimadzu, Milano, Italy): total colour (TC, AU), total polymeric pigments (TPP, expressed as % of total colour), co-pigmentation (CP, 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). Ochratoxin A was analyzed by means of an ELISA Kit (Astori, Tecnica, Italy), which identifies samples with concentrations above 2 µg/l, corresponding to the upper legal limit. Biogenic amines were determined according to the literature (Manfroi, Silva, Rizzon, Sabaini, Beatriz, & Glória, 2009). All of these 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 at 16 months (2011 and

2012 wines) and 28 months (2011 wines) after the end of the fermentation. Data are presented as mean values obtained from two replicated analyses of each duplicate vinification.

2.4. GC-MS Volatile analyses

The quali-quantitative aromatic profile of the wines produced in 2011 and 2012 was determined as follows: 20 ml of sample were combined with 100 μ l of internal standard solution (2-octanol: 500 mg/l in ethanol); after liquid-liquid extraction (Gerbi, Zeppa, & Carnacini, 1992) the volatile compounds were analyzed by injecting 1 μ l of extracted 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 μ m film thickness) (Restek, Bellefonte, PA). Chromatographic conditions were as follows: GC-grade helium as the carrier gas (flow: 1.0 ml/min), splitless injection, and detector temperature of 250°C. The following temperature gradient program 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 ionization 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 one scan per second. Identification of analyte was achieved by comparing the sample mass spectra with those of standards, NIST 2.0 (US National Institute of Standards and Technology), and/or Wiley 7 libraries. The linear retention index was calculated for each compound, which was quantified (in μ g/l) by comparing the sample's total ion current peak to that of the internal standard, corrected by the response factor of each reference standard compound to 2-octanol. When a reference standard was not available, the correction factor was calculated by means of the response factor of a compound with a similar chemical structure.

2.5. *Electronic nose*

A glass vial was filled with 10 ml of sample and held at room temperature for 60 min for equilibration. The commercial portable electronic nose PEN2 (Airsense Analytics, Milano, Italy) was used to analyze the headspace. The device is composed of an array of ten temperature-moderated metal-oxide sensors (MOS), a sampling system, a data-acquisition device and a data-processing system. The signal output of the sensors was digitized by recording and normalized to a value of 1.0 prior to sampling; this arbitrary baseline value was subtracted from the sensor responses prior to enhancement determination. A thermal desorption system ensured that sensor saturation due to ethanol was avoided. The signal output was acquired at 1-s intervals for 100 s, by which time the sensors had reached a steady state. The sensor values at 90 s were used to sort wines.

2.6. *Sensory analysis*

For each vintage (2011 and 2012), the four wines obtained from each viticultural practice (ORG and BDN) were analyzed for sensory differences six months after the end of alcoholic fermentation. For the 2011 wines the sensory analysis was also repeated in 2013 eleven months after the previous evaluation and 23 months after the end of fermentation. The panel analyzing each vintage consisted of 30 judges between 22 and 65 years of age, recruited from the staff and students in the Department of Agricultural and Food Sciences, Cesena, Italy because of their motivation and availability. The selected judges had previous experience in wine tasting and descriptive analysis. The descriptors were as follows: colour (intensity), aroma (fruit, herbaceous, spice, alcohol, overall aroma), taste (sour, bitter, alcohol, astringency, overall taste) and persistence. Four wines were

evaluated in each session. Samples (30 ml) coded with 3-digit numbers were presented at room temperature (20°C) in 170-ml tulip glasses (ISO, 1997) covered with transparent plastic dishes to preserve aroma. Samples were scored for selected descriptors on a 10-cm scale anchored with “low” and “high” intensity. Panelists were allowed to rinse their mouth by drinking water and eating unsalted crackers between samples. Tasting sessions took place in a sensory facility equipped with individual sensory booths illuminated with daylight lamps (ISO, 2007).

Wines were also evaluated for overall likeability by a panel of forty consumers (42% female and 58% male, aged between 23 and 65 years) who were requested to give a score according to preference on a 1–9 point structured scale (from “I dislike very much” to “I like very much”), as previously used by other researchers (Lawless & Heymann, 1998). For both the descriptive analysis and the preference test, the wines were presented according to a randomized complete block design.

2.7. Statistical analysis

Mean differences in chemical composition and sensory analysis of the wines were evaluated by one- and two-way (with management practice and year as factors) analyses of variance (ANOVA), and Fisher’s LSD served as a *post-hoc* test for the interaction effect. Principal component analysis (PCA) was used as an unsupervised multivariate data tool to find hidden structure among the observations. All the analyses were carried out using XLSTAT version 2011.1.05 (Addinsoft, Anglesey, UK). All statistics were performed with significance at $p \leq 0.05$.

3. Results and discussion

3.1. Chemical analyses

The differences in management (ORG or BDN) did not affect plant productivity, bunch number per plant, bunch and berry weight, or technological parameters, such as total soluble solids (Brix), pH, or total acidity for either vintage (2011 or 2012) (Botelho, Roberti, Tessarin, Garcia-Mina, & Rombolà, 2016; Picone, Trimigno, Tessarin, Donnini, Rombolà, & Capozzi, 2016). The sugar content of the grape musts ranged from 20.5 to 21.5 °Babo, considered an optimal value for Sangiovese grape maturity. The fermentation time was 14 and 18 days for vintages 2011 and 2012, respectively, regardless of the grape management practice adopted in the vineyard. The chemical analyses of the experimental wines at bottling indicated that for both vintages, biodynamic preparations did not significantly affect the following: alcohol strength, dry matter, reducing substances, optical density (at 420nm, 520nm, and 620nm), colour, hue, and total polyphenols (see **Table 1 for detailed results**).

Malic acid was not detectable in any wines due to its complete conversion to lactic acid during malolactic fermentation. No differences were recorded for the remaining parameters in the 2011 vintage, whereas in the 2012 vintage there were significant differences in total acidity (ORG was higher) and volatile acidity (BDN was higher). The pH was the only parameter significantly different in both vintages, with BDN having 0.1 unit higher than ORG. These data, combined with those presented in a previous paper by the same authors (Parpinello, Rombolà, Simoni & Versari, 2015) confirm the general trend shown during the second year of conversion from organic to biodynamic. In fact, after the first year of conversion (2009), ORG wines showed significantly higher values in: alcohol strength; volatile acidity; optical density at 420, 520, and 620 nm; colour intensity; and total polyphenols. However, after the second year of conversion (2010) the differences decreased and were limited to optical density at 420nm and total polyphenols. Moreover, the data presented in this study for 2011 and 2012 vintages show that the disparity between ORG and BDN wines decreased throughout the two years following the conversion period—in particular for chromatic components (at 420, 520, and 620nm), colour intensity, and

total polyphenols. It is noteworthy that in the 2012 vintage, BDN wines obtained higher colour intensity than ORGs, although the difference was not significant. The two-way ANOVA disclosed a significant effect of year for some parameters (**Table 1**). In particular, characteristics related to the vinification process (reducing substances, total and free SO₂) achieved higher concentrations in 2012, with the exception of volatile acidity, which was higher in 2011. In contrast, the characteristic related to grape technological ripeness (pH) was higher for the 2011 vintage. As already mentioned, shoot and bunch thinning were performed in 2011 and 2012, resulting in a lower yield (ORG/11: 2.0 kg/plant – BDN/11 2.1 kg/plant; ORG/12: 2.6 kg/plant – BDN/12: 2.6 kg/plant) and a general enhancement of wine quality (Table 1) compared to 2009 and 2010 (Parpinello, Rombolà, Simoni & Versari, 2015). Notably, both the 2011 and 2012 harvests were characterized by hot climatic conditions. However, in 2011 rains were almost absent during ripening, and a total rainfall of 204 mm was recorded from budburst to harvest; for the same phenological stage in 2012, more rain (342 mm) was recorded. As for the management practice (ORG vs BDN), the wines had similar characteristics in terms of alcohol strength, dry matter, reducing substances, free sulfur dioxide, chromatic characteristics, colour intensity, hue, total polyphenols, and lactic acid (**see details in Table 1**). Differences were found in total acidity, volatile acidity, and pH, in contrast to a lack of differences during the conversion period (Parpinello, Rombolà, Simoni & Versari, 2015). These results suggest that during this period, biodynamic management significantly reduced the majority of the basic wine characteristics (chemical and colour components) most likely as a consequence of berry composition (Picone, Trimigno, Tessarin, Donnini, Rombolà, & Capozzi, 2016). Following this period, the vine achieved a new physiological balance consequent to different canopy practices, along with the cumulative effects of soil management. First-order interactive effects between management (M) and year (Y) (**Table 1**) were limited to volatile acidity, pH, and total sulfur dioxide.

3.2. Phenolic compounds

In red wine, polyphenolics represent natural key compounds responsible for quality, sensory characteristics (astringency, bitterness, colour), and aptitude for aging. For this reason the analyses of monomeric anthocyanins, simple phenols, and phenolic acids were carried out on the wines. Profiles obtained for these experimental wines were consistent with previous compositional studies reported for Sangiovese wines (Arapitsas, Perenzoni, Nicolini, & Mattivi, 2012; Parpinello, Rombolà, Simoni & Versari, 2015). For some compounds, the main effect was ascribed to the year (**Table 2**). In fact, for delphinidin-3-glucoside, cyanidin-3-glucoside, peonidin-3-glucoside, and quercetin, concentrations were higher in 2012 (than 2011) for both vineyard management practices. The ORG/11 wine had a significantly lower concentration of (+)-catechin (18.5 mg/l) than ORG/12 (26.1 mg/l), BDN/11 (24.8 mg/l), and BDN/12 (24.9 mg/l)—whereas higher caftaric acid was characterized to BDN/12 (32.3 mg/l) compared to the other three. Results were similar for all of the other phenolics, such as petunidin-3-glucoside, malvidin-3-glucoside, gallic and coumaric acids, (–)-epicatechin, and rutin. Notably, differences in the phenolic compound concentration observed during the two years of conversion (Parpinello, Rombolà, Simoni & Versari, 2015) were not detectable in the ORG or BDN wines produced in either vintage. Trans-resveratrol, protocatechuic acid, and myricetin were below the detection limit. When management is considered as a factor (**Table 2**), no significant differences emerged between ORG and BDN wines, although the latter presented with slightly higher concentrations of malvidin-3-glucoside and (+)-catechin. With regard to vintage year, the following registered significantly higher values in 2012: delphinidin-3-glucoside, cyanidin-3-glucoside, petunidin-3-glucoside, peonidin-3-glucoside, and quercetin. The concentrations of the other phenolic compounds (malvidin-3-glucoside, gallic, coumaric, caftaric acids, (+)-catechin, (–)-epicatechin, and rutin) were similar for both years. The two-way ANOVA revealed a significant interaction between vineyard management and year for caftaric acid only ($p= 0.043$). Recently, research

comparing organic and biodynamic Lambrusco red wines obtained by stabilized, long-running management practices reported a lack of significant difference in total anthocyanins and a higher concentration of polyphenols in the biodynamic wine (Tassoni, Tango, & Ferri, 2014). Together, our previous and current results demonstrate that in four years of biodynamic management, differences in phenolic concentration progressively decreased between organic and biodynamic; in fact, the concentration of flavonoids and non-flavonoids under investigation varied according to the year of harvest, but was independent of the management practice.

3.3. Tannins and colour components

In order to get more insight into the effect of stable ORG or BDN management on colour components and their evolution over time, analyses were carried out for total anthocyanins (ANT), tannins (TN), small (SPP) and large (LPP) polymeric pigments, total iron-reactive phenols (IRP), total colour (TC), and co-pigmentation (CP) (**Table 3**). The analyses were performed in the wines at bottling (four months after the end of fermentation: denoted as ORG/11, ORG/12, BDN/11, BDN/12 and after 16 months of storage (denoted as ORG/11₍₂₀₁₃₎, ORG/12₍₂₀₁₄₎, BDN/11₍₂₀₁₃₎, BDN/12₍₂₀₁₄₎). The 2011 wines were also analyzed again after 28 months of storage (denoted as ORG/11₍₂₀₁₄₎, BDN/11₍₂₀₁₄₎). In general, a decrease of TC was registered over time, regardless of the adopted management. In wines produced in the 2011 and 2012 vintages, the TC at bottling, as well as after 16 months and 28 months of storage, was not significantly different for ORG and BDN wines. However, this similarity was not observed during the two conversion years (Parpinello, Rombolà, Simoni & Versari, 2015) as BDN wines had lower TC. The ORG and BDN management did not affect small (SPP) or large (LPP) polymeric pigments when wines stored for the same amount of time were compared, either at bottling, after 16 months, or after 28 months. Understandably, storage significantly increased the content of polymeric pigments over time. As

an example, in the ORG/11 wine, the SPP were 23.3% and LPP were 22.3%, whereas in the ORG/11₍₂₀₁₄₎—that is, after 28 months of storage—they became 30.6% and 39.5%, respectively. In the BDN/11 wine the SPP were 22.2% and LPP were 20.8%, whereas in the BDN/11₍₂₀₁₄₎ they became 30.7% and 32.1%, respectively. The same trend was observed in ORG and BDN wines produced in 2012. Accordingly, CP, ANT, TN, and IRP did not differ significantly between ORG and BDN wines for both vintages when compared at the same time of storage. Furthermore, as expected, co-pigmentation, anthocyanins, and tannins decreased over time. It is noteworthy that despite the absence of significant differences in terms of CP, BDN wines in both the 2011 and 2012 vintages showed higher values compared to ORG wines—in both fresh wines and wines after the storage period. These data run counter to the findings during the conversion period (Parpinello, Rombolà, Simoni & Versari, 2015)), during which CP was higher in ORG wines. A decrease in co-pigmentation is expected during early wine storage, especially during the first year, due to oxidation and hydrolysis cofactors (Boulton, 2001). Moreover, tannins and anthocyanins may decrease during storage, as a consequence of the degradation of anthocyanins or their incorporation into oligomeric and polymeric pigments; generally, the formation of pigmented tannin-anthocyanin polymers (LPP) is preferred over anthocyanin-acetaldehyde cross-linked oligomers and pyranoanthocyanins (SPP) (Harbertson, Picciotto, & Adams, 2003). This hypothesis is supported by the data obtained on the polymeric pigments SPP and LPP, since the pigments increased considerably throughout the storage period.

No significant differences emerged when management (M) was considered as an ANOVA factor (**Table 3**). All of the phenolic and colour components were comparable for ORG and BDN wines. The higher values of total colour, tannins, and iron-reactive phenolics which characterized ORG wines during the two years of conversion (Parpinello, Rombolà, Simoni & Versari, 2015) were not observed in the following two years when viticulture conditions had stabilized. When the 2011 and 2012 wines were compared at the time of bottling by ANOVA (**Table 3**), results indicated that the harvest year significantly affected the small polymeric pigment (SPP), whereas all of the

other parameters were similar. Over time, the colour components can either undergo modifications which are finalized with the formation of more stable compounds or degradation. As a consequence, the duration of storage can cause significant differences in these components. As expected, in the 2011 wines stored for 16 months the CP and ANT decreased, while LPP and SPP significantly increased, compared to fresh wine. A further period of storage (analyses carried out at 28 months) in 2011 wines did not significantly change the scenario. The same trend was observed in 2012 wines analyzed at bottling and after 16-months of storage: a significant decrease in CP and ANT anthocyanins and an increase in LPP large polymeric pigments and SPP (see **Table 3** for detailed results).

No significant differences were found for the analyzed characteristics (except for tannins) when the interaction between management and year (M x Y) was analyzed (**Table 3**).

3.4. Volatile compound analyses

The ORG and BDN wines produced in both vintages (2011 and 2012) were screened by electronic nose to highlight differences as a whole. When the obtained instrumental signals were analyzed by PCA (F1: 92.8%; F2: 4.8%), the wines could be clustered mainly by vintage. The loading plot showed the ORG/11 and BDN/11 wines clustered on the left side, well separated from the ORG/12 and BDN/12 wines on the right (data not shown).

The wines were also analyzed for volatile components by means of GC-MS. Overall, the differences between the 2011 and 2012 vintages of the ORG and BDN wines were significantly diminished compared to the 2009 and 2010 vintages (Parpinello, Rombolà, Simoni & Versari, 2015). The volatiles pattern profile of the latter confirmed the main effect was due to vintage (**Table 4**). In particular, 2011's ORG and BDN wines were higher in isoamyl alcohol (whiskey note), ethyl lactate (fruity, buttery, butterscotch note), and γ -butyrolactone (faint, sweet, aromatic

slightly buttery note) whereas the 2012 wines contained significantly higher concentrations of isobutyl alcohol (wine-like note), 1-hexanol (vegetal), isoamyl acetate (banana), ethyl caproate (pineapple, banana note), ethyl 3-hydroxybutyrate (fresh fruit, grape note), hexanoic acid (fruity), octanoic acid (fruity, floral, apricot note), 3-(methylthio) propanol (sweet note), and 2H-pyran-2,6(3H) dione (coconut, sweet). The only significant difference between ORG and BDN was diethyl succinate (fruity note), which in the 2012 vintage reached a higher concentration in BDN wines.

3.5. Sensory evaluation

Sensory evaluation of the wines was based on the descriptors reported in the Materials and methods section. In all vintages, replicates of the same management (ORG and BDN) were not significantly different from each other; therefore, the results are presented as mean values of the sensory data obtained in the evaluation of every vinification for each management. Wines produced in 2011 (ORG/11 and BDN/11) were not significantly different in any of the scored descriptors. However, note that the fruity note was higher in BDN/11 wine compared to ORG/11, whereas acidity was higher in ORG wine. The sensory evaluation of the same wines carried out in 2013 (after one year of storage) confirmed the results obtained in 2012, although no significant differences in terms of astringency (BDN/11: 4.6; ORG/11: 5.2; $p=0.110$) or alcohol by taste (BDN/11: 4.2; ORG/11: 4.9; $p=0.089$) were found. The latter result was consistent with the chemical analyses. Wines produced in 2012 were characterized by a significant difference in terms of astringency (BDN/12: 5.1; ORG/12: 6.0; $p=0.010$), odor complexity (BDN/12: 4.8; ORG/12: 5.4; $p=0.038$), and—to a lesser extent—alcohol by taste (BDN/12: 4.6; ORG/12: 5.0; $p=0.099$) and persistency (BDN/12: 5.1; ORG/12: 5.6; $p=0.099$). Consumers liked the wines from both vintages equally. A general overview of the sensory results is represented by means of the PCA, which explained 71.7% of the

variability with the first two components ($F_1 = 52.7\%$; $F_2 = 19.0\%$) (**Figure 1**). The ORG/11 and BDN/11 were characterized by a “tasting year” effect, with higher values obtained in evaluations carried out in 2012 (right side of the bi-plot). Conversely, wines produced in 2012 were positioned in opposite quadrants of the plot (upper/right for ORG/12 and lower/left for BDN/12). A separation between ORG and BDN wines in the bi-plot can be clearly visualized, with ORG wines occupying the upper part of the bi-dimensional space. These results suggest that although very few significant differences were found when only single sensory descriptors were considered, a multivariate analysis of all available sensory data revealed an effect of biodynamic management on the sensory characteristics of the wines.

3.6. *Ochratoxin A and biogenic amines*

The concentrations of ochratoxin A were below the detection limit (LOQ: 2 $\mu\text{g/l}$). Similar results were obtained with biogenic amines (putrescine, cadaverine, tyramine, methylamine, agmatine, spermine, spermidine, histamine): values were below the limit of 2–10 mg/l (a limit recommended by several international control committees, such as the International Committee on Food Microbiology and Hygiene (ICFMH), European Commission (EC)).

4. Conclusions

In this two-year study of 2011 and 2012 vintages we contribute to the understanding of the effect of two sustainable management practices (biodynamic and organic) on the chemical composition and sensory characteristics of Sangiovese red wines. In a previous publication (Parpinello, Rombolà, Simoni & Versari, 2015) we showed that during the two-year conversion period (2009 and 2010)

from organic to biodynamic in the same vineyard, the wine quality was greatly affected by the biodynamic 'preparations' but not conditioned by the year. Results from the present study confirm the trend observed during the second year of conversion (2010). The differences between ORG and BDN wines tended to disappear; the wines were characterized by similar chemical and sensory features. These data were confirmed by sensory tests carried out with panelists and less experienced consumers. The estimated increase in cost due to the utilization of biodynamic preparations and their application in the vine for the production of biodynamic grape was 5% per liter. In the cellar, the application of similar vinification protocols for ORG and BDN wines production did not create any cost differences. Following our results it is possible to hypothesize that, initially, biodynamic management can induce changes in berry composition ensuing from modifications in the plant and the soil. Then, the vine achieves a new physiological balance consequent to different canopy practices along with the cumulative effects of biodynamic soil management, which together ensure the production of high quality Sangiovese grapes and wines.

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Table 1. Mean separation of basic chemical composition (one-way and two-way ANOVA) of Sangiovese red wines obtained from grapes harvested during the 2011 and the 2012 season from vineyards managed with organic (ORG) or biodynamic (BDN) systems and the interactive effect of both factors.

Table 2. Mean separation and factor interaction (one-way and two-way ANOVA) of anthocyanin and phenolic compositions (mg/l) of Sangiovese red wines obtained from grapes managed with organic (ORG) or biodynamic (BDN) systems during the 2011 and the 2012 season.

Table 3. One-way and two-way ANOVA of the phenolic and color components of ORG and BDN Sangiovese red wines. Data represent the mean value of two vinifications.

Table 4. Volatile compositions of ORG and BDN Sangiovese red wines ($\mu\text{g/l}$) produced during the 2011 and 2012 harvests (mean value of two vinifications).

Figure 1. Bi-plot (score and loading) of the ORG and BDN wines produced in 2011 and 2012 clustered according to sensory characteristics

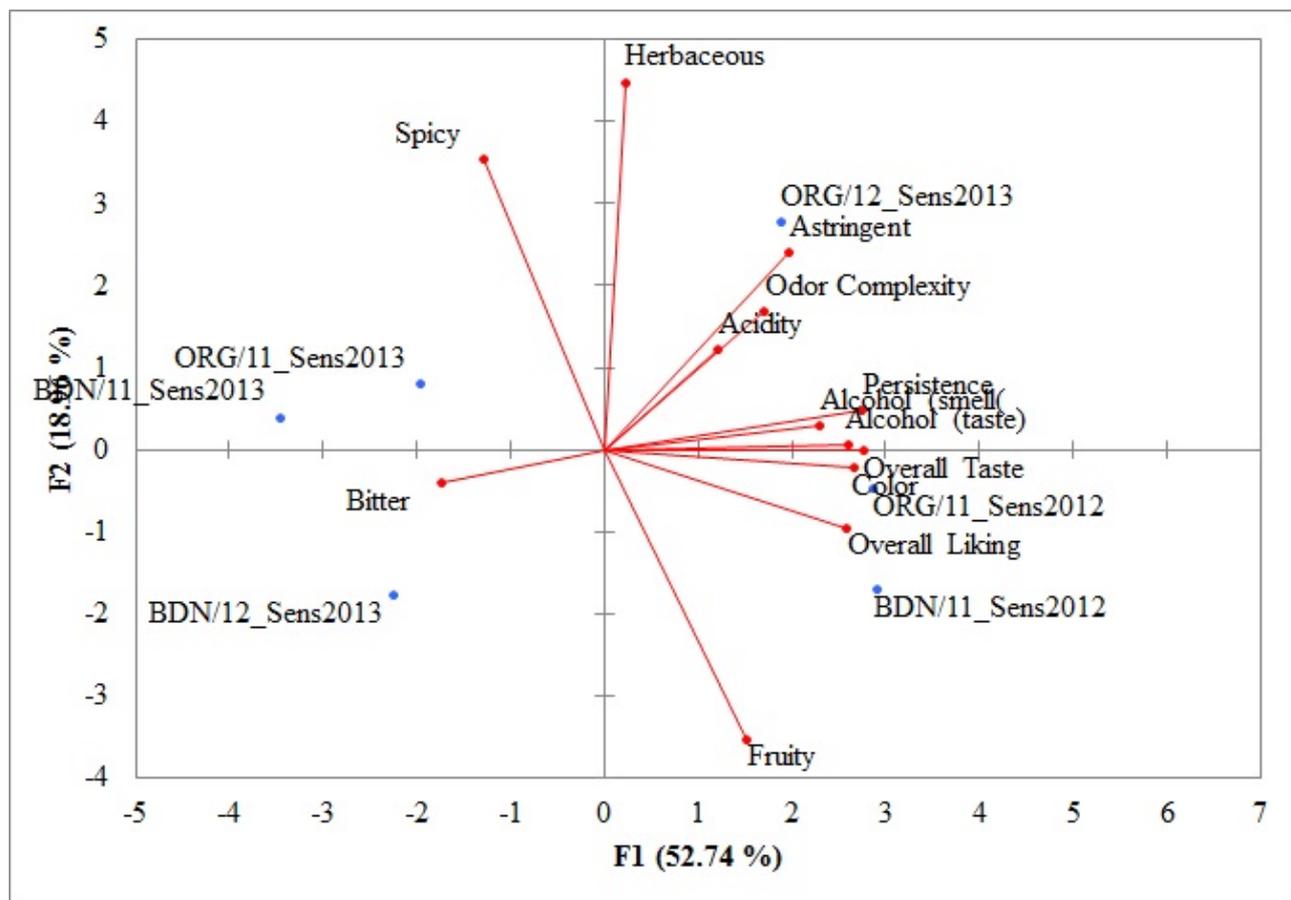


Table 1.

	ALC (%)	TA (g/l)	VA (g/l)	pH	DM (g/l)	RS (g/l)	SO ₂ T (mg/l)	SO ₂ F (mg/l)	OD 420 (AU)	OD 520 (AU)	OD 620 (AU)	CI (AU)	HUE (AU)
1	14.0 ^a	6.2 ^{ab}	0.46 ^a	3.4 ^b	25.9 ^{ab}	1.5 ^a	61 ^b	26 ^b	2.92 ^a	4.47 ^a	0.93 ^a	8.3 ^a	0.65 ^a
2	14.7 ^a	6.8 ^a	0.21 ^c	3.3 ^c	29.2 ^a	1.9 ^a	67 ^b	34 ^a	2.97 ^a	4.63 ^a	0.87 ^a	8.5 ^a	0.64 ^a
1	13.1 ^a	5.5 ^b	0.44 ^a	3.5 ^a	25.0 ^a	1.4 ^a	65 ^b	26 ^b	2.76 ^a	3.74 ^a	0.83 ^a	7.3 ^a	0.69 ^a
2	13.7 ^a	5.7 ^b	0.36 ^b	3.4 ^b	25.9 ^{ab}	1.6 ^a	94 ^a	32 ^{ab}	2.94 ^a	5.21 ^a	0.79 ^a	8.9 ^a	0.56 ^a
G	14.4 ^a	6.5 ^a	0.33 ^b	3.3 ^b	27.5 ^a	1.7 ^a	64 ^b	30 ^a	2.95 ^a	4.55 ^a	0.90 ^a	8.4 ^a	0.65 ^a
N	13.4 ^a	5.6 ^b	0.40 ^a	3.5 ^a	25.4 ^a	1.5 ^a	80 ^a	29 ^a	2.85 ^a	4.47 ^a	0.81 ^a	8.1 ^a	0.63 ^a
1	13.5 ^a	5.8 ^a	0.45 ^a	3.5 ^a	25.4 ^a	1.5 ^b	63 ^b	26 ^b	2.84 ^a	4.10 ^a	0.88 ^a	7.8 ^a	0.67 ^a
2	14.2 ^a	6.2 ^a	0.28 ^b	3.4 ^b	27.5 ^a	1.8 ^a	81 ^a	34 ^a	2.95 ^a	4.92 ^a	0.83 ^a	8.7 ^a	0.60 ^a
ue	0.957	0.409	0.001	0.018	0.303	0.570	0.033	0.595	0.777	0.215	0.828	0.312	0.142

Legend: Y: year; M: management; ORG: organic management; BDN: biodynamic management; ALC: alcohol strength; TA: titratable acidity; VA: volatile acidity; DM: total dry matter; RS: reducing sugars; SO₂T: total sulphure dioxide; SO₂F: free sulphure dioxide; OD: optical density; CI: color intensity; HUE: color hue; TP: total polyphenols; LA: lactic acid. M x Y: management x year interaction effect. Unless specified, each value is the mean of two independent vinifications of 200 Kg (replicates). Analyses were performed at bottling (4 months after the end of fermentation). The superscript letters represent the results of Fisher's LSD comparison tests: for values with the same letter, different wines have significantly different means ($\alpha = 0.05, 0.1$). Values for factors with a significant ($p \leq 0.05$) interaction effect are shown in bold.

Table 2.

Y/ M	Anthocyanins					Phenolic acids	Cinnamic acids		Flavanols		Flavonols
	Df-3-glc	Cn-3-glc	Pt-3-glc	Pn-3-glc	Mv-3-glc	Gallic	Cout	Caft	(+)-Cat	(-)-Epic	Rut
2011	5.8 ^b	2.9 ^b	8.3 ^a	4.2 ^b	26.6 ^a	27.0 ^a	12.9 ^a	29.0 ^{ab}	18.5 ^b	4.2 ^a	39.5 ^a
2012	12.2 ^a	7.1 ^a	15.5 ^a	9.1 ^a	38.8 ^a	28.8 ^a	13.2 ^a	28.6 ^b	26.1 ^a	2.9 ^a	33.2 ^a
2011	5.6 ^b	2.3 ^b	8.6 ^a	3.8 ^b	32.7 ^a	25.6 ^a	12.5 ^a	27.3 ^b	24.8 ^a	2.3 ^a	37.1 ^a
2012	12.0 ^a	7.0 ^a	15.2 ^a	9.1 ^a	38.5 ^a	29.2 ^a	12.7 ^a	32.3 ^a	24.9 ^a	3.1 ^a	34.0 ^a
t ORG	9.0 ^a	5.0 ^a	11.9 ^a	6.7 ^a	32.7 ^a	27.9 ^a	13.0 ^a	28.8 ^a	22.3 ^a	3.5 ^a	36.3 ^a
	BDN	8.8 ^a	4.7 ^a	12.0 ^a	6.5 ^a	35.6 ^a	12.6 ^a	29.8 ^a	24.8 ^a	2.7 ^a	35.5 ^a
2011	5.7 ^b	2.6 ^b	8.6 ^b	4.1 ^b	29.7 ^a	26.3 ^a	12.7 ^a	28.1 ^a	21.6 ^a	3.3 ^a	38.3 ^a
2012	12.1 ^a	7.1 ^a	15.4 ^a	9.1 ^a	38.7 ^a	29.0 ^a	12.9 ^a	30.4 ^a	25.5 ^a	3.0 ^a	33.6 ^a
<i>p-value</i>	0.997	0.757	0.860	0.853	0.698	0.778	0.968	0.043	0.067	0.333	0.698

Legend: Y: year; Mng: management; Df: delphinidin; Cn: cyanidin; Pt: petunidin; Pn: peonidin; Mv: malvidin; glc: glucoside; Cout: coumaric acid; Caft: caftaric acid; Cat: catechin; Epic: epicatechin; Rut: rutin; Quer: quercetin. Unless specified, each value is the mean of two independent vinifications of 200 Kg (replicates). Mng x Y: interaction effect of management x year. The superscript letters represent the results of Fisher's LSD *post hoc* comparison tests: for values with the same letter, different wines have significantly different means ($\alpha=0.05, 0.1$). Significant values ($p \leq 0.05$) are shown in bold.

Table 3.

ANOVA factor	Year of harvest (year of analyses) /M	TC (AU)	TTP (%)	CP (%)	ANT (%)	LPP (%)	SPP (%)	TN (mg/l)	IRP (mg/l)
ORG	2011	4.3 ^a	45.6 ^c	12.6 ^{abc}	41.7 ^a	22.3 ^{cd}	23.3 ^{de}	953 ^{ab}	2249 ^{ab}
	2012	4.1 ^{ab}	38.0 ^{cd}	19.9 ^a	42.1 ^a	18.9 ^d	19.1 ^f	1129 ^a	2593 ^a
BDN	2011	3.9 ^{abc}	43.0 ^{cd}	18.6 ^{ab}	38.4 ^{ab}	20.8 ^d	22.2 ^e	874 ^{abc}	2120 ^{ab}
	2012	4.1 ^{ab}	35.0 ^d	20.8 ^a	44.2 ^a	17.2 ^d	17.8 ^f	835 ^{abc}	2167 ^{ab}
ORG	2011 ₍₂₀₁₃₎	3.3 ^d	67.6 ^{ab}	4.3 ^d	28.0 ^e	32.5 ^{ab}	35.1 ^a	814 ^{bc}	2185 ^{ab}
	2011 ₍₂₀₁₄₎	3.4 ^d	70.1 ^a	4.5 ^{cd}	25.4 ^e	39.5 ^a	30.6 ^b	641 ^c	1937 ^b
	2012 ₍₂₀₁₄₎	3.8 ^{bc}	64.4 ^{ab}	6.9 ^{cd}	28.5 ^e	37.2 ^{ab}	27.2 ^c	884 ^{abc}	2112 ^{ab}
BDN	2011 ₍₂₀₁₃₎	3.2 ^d	60.4 ^b	8.1 ^{cd}	31.4 ^{bc}	29.8 ^{cb}	30.6 ^b	825 ^{bc}	2054 ^b
	2011 ₍₂₀₁₄₎	3.2 ^d	62.8 ^{ab}	7.5 ^{cd}	29.7 ^e	32.1 ^{ab}	30.7 ^b	607 ^c	1848 ^b
	2012 ₍₂₀₁₄₎	3.5 ^{cd}	61.6 ^{ab}	10.9 ^{bed}	27.4 ^e	35.6 ^{ab}	26.0 ^{cd}	856 ^{abc}	2038 ^b
Management	ORG	3.8 ^a	57.1 ^a	9.6 ^a	33.1 ^a	30.0 ^a	27.1 ^a	884 ^a	2215 ^a

	BDN	3.6 ^a	52.6 ^a	13.2 ^a	34.2 ^a	27.1 ^a	25.4 ^a	800 ^a	2045 ^a
	2011	4.1 ^a	44.3 ^b	15.6 ^{ab}	40 ^a	21.5 ^b	22.7 ^c	914 ^a	2184 ^{ab}
Year (Y)	2011 ₍₂₀₁₃₎	3.3 ^b	64.0 ^a	6.2 ^c	29.7 ^b	31.2 ^a	32.8 ^a	820 ^{ab}	2119 ^{ab}
	2011 ₍₂₀₁₄₎	3.3 ^b	66.5 ^a	6.0 ^c	27.5 ^b	35.7 ^a	30.7 ^a	624 ^b	1892 ^b
	2012	4.1 ^a	36.5 ^b	20.3 ^a	43.1 ^a	18.0 ^b	18.5 ^d	982 ^a	2380 ^a
	2012 ₍₂₀₁₄₎	3.7 ^{ab}	63.1 ^a	8.9 ^{cb}	28.0 ^b	36.5 ^a	26.6 ^b	871 ^a	2075 ^{ab}
M x Y	<i>p-value</i>	0.684	0.840	0.898	0.550	0.801	0.151	0.002	0.787

Legend: Y: year; Mng: management. Year of harvest (year of analyses): as an example, ORG 2011₍₂₀₁₂₎ refers to organic wine produced in harvest 2011 and analyzed at bottling in 2012 (4 month after the end of fermentation), TC: total color; TPP: total polymeric pigments; CP: copigmentation; ANT: anthocyanins; LPP: large polymeric pigments; SPP: small polymeric pigments; TN: tannins; IRP: iron reactive phenolics. Unless specified, each value is the mean of two independent vinifications of 200 Kg (replicates). Mng x Y: interaction effect of management x year. The superscript letters represent the results of Fisher's LSD *post hoc* comparison tests: for values with the same letter, different wines have significantly different means ($\alpha=0.05$ or 0.1). Significant values ($p \leq 0.05$) are shown in bold.

Table 4.

Compound ($\mu\text{g/l}$)	Wine			
	ORG/11	ORG/12	BDN/11	BDN/12
Alcohols				
Isobutyl alcohol***	327b	50374 ^a	420 ^b	43577 ^a
<i>n</i> Butanol	513	518	499	451
Isoamyl alcohol*	88960 ^a	51450 ^b	78520 ^a	42069 ^b
2-Hexanol**	131 ^c	372 ^a	137 ^{bc}	335 ^{ab}
4-Methyl-1-pentanol	nd	57	nd	48
3-Methyl-1-pentanol***	8 ^b	201 ^a	34 ^b	191 ^a
1-Hexanol**	226 ^b	1082 ^a	101 ^b	1095 ^a
<i>t</i> -3-Hexen-1-ol	nd	7	nd	9
<i>c</i> -3-Hexen-1-ol	nd	8	nd	11
3-Ethoxy-1-propanol	nd	79	nd	97
2,3-Butanediol	21114	15320	17774	13878
<i>n</i> -Dodecan-1-ol	16	nd	10	nd
Esters				
Isoamyl acetate**	760 ^b	1313 ^a	735 ^b	1149 ^{ab}
Ethyl caproate***	100 ^b	390 ^a	73 ^b	384 ^a
Ethyl lactate***	51160 ^a	12414 ^b	50036 ^a	21922 ^b

Ethyl caprylate**	nd	474 ^b	nd	526 ^a
Ethyl 3-hydroxybutyrate***	233 ^b	787 ^a	255 ^b	805 ^a
Ethyl decanoate	41	nd	44	nd
Diethyl succinate*	4768 ^{ab}	1480 ^b	5432 ^a	6468 ^a
β -Phenylethyl acetate**	nd	46 ^a	nd	29 ^b
N-acetylglycine ethyl ester	78	nd	55	nd
Butanedioic acid, monoethyl ester	226888	137384	244484	238727
Acids				
Acetic acid	13110	4205	9611	6402
Isobutyric acid	1744	1692	1502	1697
n-Butyric acid	nd	448	nd	509
Pentanoic acid + dioxane**	2588 ^a	1395 ^{ab}	nd	766 ^b
Hexanoic acid**	910 ^b	1618 ^a	882 ^b	1782 ^a
Octanoic acid*	853 ^b	1823 ^a	988 ^{ab}	1850 ^a
Decanoic acid**	118 ^b	414 ^a	140 ^b	347 ^{ab}
Dodecanoic acid	nd	158	nd	153
Hexadecanoic acid**	112 ^{cb}	380 ^a	34 ^c	314 ^{ab}
Miscellaneous				
3-(Methylthio) propanol**	520 ^b	900 ^a	521 ^b	838 ^a
β -Phenylethyl alcohol	32679	27151	34162	25913
3-Hydroxy-2-butanone	6065	888	8629	3447
γ -Butyrolactone*	29950 ^a	15971 ^b	25904 ^{ab}	14679 ^b
4-Hydroxy-2-butanone***	291 ^b	804 ^a	348 ^b	867 ^a
N-(3-Methylbutyl) Acetamide	nd	27	nd	9
1,4-Diacetoxybutane + benzyl alcohol**	120 ^b	204 ^{ab}	140 ^b	287 ^a
2H-pyran-2,6(3H) dione**	110 ^b	323 ^a	110 ^b	336 ^a
Benzaldehyde 4-pentyl	176	310	160	306
Dihydro-5-(1-hydroxyethyl) -2-(3H) furanone**	454 ^b	564 ^b	1065 ^{ab}	2267 ^a

1H-Indole-3-ethanol	nd	2108	nd	3125
4-Hydroxy-benzeneethanol**	7445 ^{ab}	19623 ^a	5505 ^b	16471 ^{ab}
4-Hydroxy-3-methoxy- acetophenone	26 ^b	99 ^a	18 ^b	95 ^a

Each value is the mean of two independent vinifications of 200 Kg (replicates). The superscript letters represent the results of Fisher's LSD *post hoc* comparison tests: for values with the same letter, different wines have significantly different means; * $p \leq 0.10$; ** $p \leq 0.05$; *** $p \leq 0.001$; nd: not detected.

Highlights

- Biodynamic: a management for the production of quality wines
- Chemical characteristics of wine can be affected by the management system as well as the year of the harvest
- After two years conversion biodynamic wines are similar to organic wines for chemical and sensory characteristics