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Comparison of biogenic amine and polyphenol profiles of grape berries and wines obtained following conventional, organic and biodynamic agricultural and oenological practices

Annalisa Tassoni *, Nunzio Tango, Maura Ferri

Department of Biological, Geological and Environmental Sciences, University of Bologna, Via Irnerio 42, 40126 Bologna, Italy

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ABSTRACT

The bio-active compounds present in food and beverages have a high potential influence on the future health of humans. The levels of biogenic amines, anthocyanins, polyphenols and antioxidant activity were measured in white (Pignoletto) and red (Sangiovese) grape berries and wines from the Emilia-Romagna region (Italy) obtained following conventional, organic and biodynamic agricultural and oenological practices. No significant difference was shown among the samples coming from different agricultural and winemaking practices. Principal Component Analysis was also performed. Biogenic amine amounts were higher in red than in white berries, while in the wines an opposite trend was observed, with histamine, tyramine and putrescine being the most abundant in Pignoletto wines. Red grapes and wines were richer in anthocyanins and showed higher antioxidant activity than white ones. The total level of polyphenols was similar in red and white berries, but with different metabolite profiles depending on the grape variety.

1. Introduction

The bio-active nutraceutical and anti-nutraceutical compounds present in food and beverages have a potential influence on human health (or poor health), however, with very few exceptions, the molecular composition and complexity of foods cannot as yet be fully described.

Among the anti-nutraceutical compounds, amines are basic nitrogenous compounds synthesised by metabolic pathways in plant and mammalian cells that usually involve decarboxylation of precursor amino acids (Beneduce et al., 2010; Kusano, Berberich, Tateda, & Takahashi, 2008). The term "biogenic amines" includes decarboxylation products such as histamine (HIM), serotonine, tyramine (TYM), tryptamine (TRYPT), phenylethylamine but also aliphatic polyamines such as agmatine, putrescine (PUT), cadaverine (CAD), spermidine (SPD) and spermine (SPM).

In food and beverages, biogenic amines are formed by the enzymes from raw material or are generated by microbial decarboxylation of amino acids and in particular they are present in all those foods that are produced by fermentation processes such as cheese, wine, beer, sauerkraut (EFSA Panel on Biological Hazards, 2011). Some types of biogenic amines (such as HIM, TYM, TRYPT, PUT and CAD) are undesirable in food and beverages because, if absorbed at too high concentrations, they may cause headaches, respiratory distress, heart palpitation, hypertension or hypotension, and several allergenic disorders (EFSA Panel on Biological Hazards, 2011). Aliphatic polyamines, such as PUT, SPD and SPM, are essential for normal cell growth but also, at high concentrations, may sustain cancer cell proliferation (EFSA Panel on Biological Hazards, 2011). Therefore assessing the everyday dietary intake of biogenic amines could represent an important way of reducing their level in the body pool.

The identification of different amines in wine samples has been carried out in several investigations. More than twenty amines have been identified in wines and their total concentration has been reported to range from a few to about 50 mg/L, depending on many factors including the wine making conditions, must fermentation and ageing. HIM, TYM and PUT are the most significant biogenic amines encountered in wines (Beneduce et al., 2010; EFSA Panel on Biological Hazards, 2011; Mafra, Herbert, Santos, Barros, & Alves, 1999).

Over the last decade the beneficial influence on health of a moderate wine consumption has been increasingly investigated. The health-protective properties of grapes and wines are attributed to their antioxidant activities, i.e. their capability to eliminate reactive oxygen species (ROS). Consequently, numerous papers have focused on the determination of the antioxidant capacity of grapes and wines, as well as on the content of their polyphenols which are largely responsible for the antioxidant action (Minussi et al., 2003; Urquiaga & Lieghton, 2005).

Grape polyphenols, such as flavonoids (i.e. catechins and anthocyanins) and stilbenes (i.e. resveratrol (RESV)), are one of the most

* Corresponding author. Tel.: +39 051 2091280; fax: +39 051 242576.
E-mail address: annalisa.tassoni2@unibo.it (A. Tassoni).

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widespread groups of plant metabolites synthesised through the very complex phenylpropanoid pathway (Iriti & Faoro, 2004). Flavonoids and stilbenes occur both as glycosides and aglycones (Bavaresco, Fregoni, van Zeller de Macedo Basto Gançalves, & Vezzulli, 2009) and, after ingestion through daily diet, are absorbed by the small intestine mucosa increasing the antioxidant capacity of blood and aiding in the prevention of cancer and cardiovascular diseases (King, Bomser, & Min, 2006; Urquiaga & Lieghton, 2005). Among flavonoids, catechins are antioxidant metabolites particularly abundant in wines (Iriti & Faoro, 2004).

Great attention has been given to the stilbene family and in particular to RESV due to its healthy properties (Delmas, Lancon, Colin, Jannin, & Latruffe, 2006; King et al., 2006). Its mono-glucosylated derivatives piceid (PIC) and resveratroloside (RDE) are present at high levels in grape berries and wines and possess antioxidant activity comparable to free RESV but, due to the presence of the glucose residue, they have a more extended half-life and bioavailability (Regev-Shoshani, Shoseyov, Bilkis, & Kerem, 2003). In addition, piceatannol (PICEAT) is a naturally occurring derivative of RESV (with four –OH groups) synthesised in grape berries only during ripening (Bavaresco et al., 2003) and was shown to inhibit the proliferation of cancer cell lines via apoptosis and cell cycle arrest (King et al., 2006).

Interestingly the interaction between polyphenols and biogenic amines metabolic pathways has been pointed out. Catechins were shown to target some enzymes of biogenic amine biosynthetic pathways (Melgarejo, Urdiales, Sánchez-Jiménez, & Medina, 2010). In particular epigallocatechin gallate (EGCG) specifically reduces HIM and PUT production by inhibiting histidine decarboxylase (HDC) and ornithine decarboxylase activities, while enhancing the activity of SPD/SPM N^1-acetyltransferase (SSAT) enzyme that promotes polyamine catabolism (Kusano et al., 2008; Melgarejo et al., 2010; Nitta, Kikuzaki, & Ueno, 2007). Studies performed with Caco-2 colorectal cancer cells, related the presence of RESV and its natural derivative PICEAT, with the modulation of enzymes involved in the biosynthesis and catabolism of amines, confirming a possible chemopreventive effect of stilbenes (Wolter, Ulrich, & Stein, 2004).

Following previous considerations it should be useful to actively promote the production of functional beverages and foods, having a modified balance between amines and polyphenols, by using different agricultural management practices and processing methods. It is in fact well known that the amount and spectrum of nutrients and metabolites in food and beverages not only depends upon their processing and storage methods but also is largely influenced by the farming system with which the raw materials are produced. Several published papers aimed to compare the metabolite profile of several crops grown under conventional, organic and biodynamic agricultural practices. In general, organic products are perceived by the public as healthier and safer than those produced through conventional agriculture. There are fundamental differences in organic and conventional production practices, but limited information is available on how these influence the nutritional quality of food. Research data showed that some crops grown under organic farming practices contained more bioactive substances such as flavones, vitamin C, carotenoids and total polyphenols. Some studies confirmed better biological activity of organic products versus conventional due to the higher content of bioactive compounds (Asami, Hing, Barret, & Mitchell, 2003; Olsson, Andersson, Oredsson, Berglund, & Gustavsson, 2006). Conversely, other researches demonstrated no significant difference between general the metabolic profile, phenolic levels and nutritional values of buckwheat groats (Kalinova & Vrchotova, 2011), wheat grains (Zörb, Langenkämper, Betsche, Niehaus, & Barsch, 2006) and apples (Valavanidis, Vlachogianni, Psomas, Zovoilli, & Siatis, 2009) grown under conventional and organic farming.

Biodynamic farming is similar in many ways to the better-known organic agriculture. Both use composting and cover cropping instead of mineral fertilising, and ban pesticides, herbicides, hormones and other chemicals. The difference from organic agriculture, apart from philosophical and historical aspects, lies in the use of biodynamic preparations which contain specific herbs or minerals, treated or fermented with animal organs. These preparations are applied in homoeopathic form, generally as field sprays after dynamisation. The different types and aims of biodynamic preparations have been described and are supposed to lie in the improvement of soil and crop quality (Reeve et al., 2005). One study on wine grape quality showed no differences in leaf and grape analysis, and only in one year out of seven of vintage was a higher content of polyphenols and anthocyanins found in biodynamically-cultivated grape with respect to organically-cultivated plants (Reeve et al., 2005).

Up to now, no information is available about the variation of biogenic amine levels in crops, and in particular grape, grown by using different management systems.

The public opinion generally considers organic and biodynamic foods healthier than the conventional ones, however the scientific evidence is still poor and ambiguous. In this view, the present study aims to compare conventional, organic and biodynamic white and red grapes and the related wines, to ascertain whether the different agricultural practices and winemaking procedures, may directly influence the profiles and contents of biogenic amines and polyphenols, and the antioxidant capacity.

2. Materials and methods

2.1. Materials

Grape berries of *Vitis vinifera* var. Pignoletto (white, autochthonous variety) and Sangiovese (red, international variety) and the derived wines were collected in 2009 from producers of the Emilia-Romagna region (Italy). Pignoletto and Sangiovese grapevines were grown by using the following agricultural practices: conventional (Pignoletto from Vigneto San Vito, Monteveglio, Bologna and Sangiovese from Antonio Gallegati, Tebano, Faenza, Ravenna), organic (Pignoletto from Maria Bortolotti, Zola Predosa, Bologna and Sangiovese from Quinzân, Faenza, Ravenna) and biodynamic (Pignoletto from Vigneto San Vito, Monteveglio, Bologna and Sangiovese from Paolo Francesconi, Faenza, Ravenna).

The berries (white or red) were harvested during vintage time on the same day, picking bunches from different plants grown in different vineyard areas, and at different light/shadow exposure. About 10 kg of grape were collected for each vineyard, immediately frozen with liquid nitrogen and stored at −80 °C. The grapes were successively ground in liquid nitrogen and the powders, stored at −80 °C, were used for the following analyses. Wines were produced from grapes on site by the same producers/wineries according to the relative conventional, organic and biodynamic technical regulations (see winemaking parameters in Supplementary Table 1). The wines were collected from wineries at the end of the production process, immediately after bottling, centrifuged at 13,000g for 10 min to remove solid residues and immediately stored at −20 °C until analysis.

2.2. Quantification of biogenic amines

Free biogenic amines (tryptamine, histamine, tyramine, diamine-propane, cadaverine, putrescine, spermidine and spermine) analyses were performed (Tassoni, van Buuren, Franceschetti, Fornalè, & Bagni, 2000). The grape samples (about 0.2 gFW of powders) were homogenised in 10 volumes of 4% (v/v) cold perchloric acid and centrifuged at 20,000g for 30 min at 4 °C and the supernatant was used for free amine determination. Aliquots (0.2 ml) of supernatants or of wines were derivatised with dansyl-chloride (3 mg/ml of acetone), extracted with toluene and analysed by high-performance liquid chromatography (HPLC, Jasco, Großumstad, Germany; equipped with an on-line spectrofluorometer Jasco 821-FP) with a reverse phase C18 column (Gemini, 5 μM particle diameter, 4.6 × 250 mm, Phenomenex, Torrance, CA, USA) (Tassoni et al., 2000). The solvent gradient (1 ml/min) was as follows: 0 min acetonitrile (ACN):H_2O (60/40 v/v); 5.5 min ACN:H_2O (70/30 v/v); 7 min ACN:H_2O (80/20 v/v); 9 min ACN:H_2O (100/0 v/v); 11 min ACN:H_2O (100/0 v/v); 13 min ACN:H_2O (70/30 v/v); 16 min ACN:H_2O (60/40 v/v); 21 min ACN:H_2O (60/40 v/v).

2.3. Total polyphenol quantification

Total polyphenols were determined by using the Folin–Ciocalteu method (Singleton, Orthofer, & Lamuela-Raventos, 1999). Grape powder samples (0.5 gFW) were extracted by overnight shaking at 4 °C with 4 ml of 98:2 methanol: 12 N HCl and centrifuged 5000g for 15 min at 4 °C. A suitable volume of grape methanolic extracts or of wines was diluted to 1.6 ml with water and 100 μL of Folin–Ciocalteu reagent were added. After 5 min the reaction was stopped with 300 μL of 20% (w/v) sodium carbonate. The mixture was vortexed for 15 s and incubated at 40 °C for 30 min in the dark, before measuring the absorbance at 765 nm. The results were expressed as gallic acid (GA) equivalents by means of a calibration curve.

2.4. Quantification of total anthocyanins

Anthocyanins were extracted from grape powders (0.5 gFW) which were resuspended in 4 ml of extraction solution (98:2 methanol: 12 N HCl) and incubated at 65 °C for 2 h. After centrifugation for 10 min at 4500g at room temperature, suitable volumes of supernatants (grape extracts) and of wines were used for spectrophotometric analyses. Absorbance (Abs) was measured for each sample at 530 and 657 nm and the anthocyanin absorbance was calculated as $\Delta Abs_{anthocyanins} = Abs_{530} - (0.25\ Abs_{657})$. Abs_{657} was used to correct for the presence of chlorophyll degradation products such as pheophytins (Ferri et al., 2009).

2.5. Quantification of polyphenols by HPLC

Polyphenols were extracted from about 0.5 gFW of grape powders (incubated overnight with 5 ml of 95% v/v methanol) and from 5 ml of wines. The samples were loaded onto a Strata-X column (33 mm polymeric sorbent 60 mg in 3 ml, Phenomenex, Torrence, CA, USA) and polyphenols were eluted by 100% v/v methanol, completely dried and resuspended in 200 μL of 1:9 ACN/0.2% v/v acetic acid before being directly injected into HPLC-DAD (column Gemini C18, 5 μm particles 250 × 4.6 mm, pre-column SecurityGuard Ea, Phenomenex, Torrence, CA, USA) equipped with an on-line diode array detector (MD-2010, Plus, Jasco Instruments, Großumstad, Germany), as described by Ferri et al. (2009). The adopted HPLC-DAD separation procedure allowed the simultaneous analysis of the following compounds: (+)-catechin (CAT), (−)-epicatechin (EC), (−)-epigallocatechin-gallate (EGCG), epigallocatechin (EGC), epicatechin-3-gallate (ECG), *trans*- and *cis*-resveratrol (RESV), *trans*- and *cis*-resveratroloside (RDE), *trans*- and *cis*-piceid (PIC), piceatannol (PICEAT), quercetin (QUERC), rutin (RUT), (±)-naringenin, myricetin (MYR), vanillin (VAN), gallic acid (GA) and *trans*-cinnamic, *p*-coumaric, caffeic, ferulic, sinapic and chlorogenic acids. The HPLC standards were purchased from Sigma–Aldrich (Milano, Italy) except for *cis*-RESV, *trans*- and *cis*-RDE, *trans*- and *cis*-PIC which were obtained as reported by Ferri et al. (2009).

2.6. Determination of antioxidant activity by DPPH method

Antioxidant activity was measured using the method based on the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging capacity (Brand-Williams, Cuvelier, & Berset, 1995), with minor modifications. Aliquots of ascorbic acid (AA) standard solution, grape methanolic extracts (see paragraph 2.3) or wine samples, were added to 0.5 ml of 90 μM DPPH solution (dissolved in methanol) and the total reaction volume was taken up to 1 ml with 95% (v/v) methanol. The mixture was vortexed for 15 s and left to stand at room temperature for 30 min in the dark, before measuring the absorbance at 517 nm. The results were expressed as AA equivalents by means of the dose–response calibration curve.

2.7. Statistical analyses and Principal Component Analysis

Two independent replicates were performed for all experiments and the relative extracts were analysed in technical duplicates. The presented results are the means of the four data ($n = 4$) ±SE.

The quantitative mean data obtained from the different metabolite determinations were used to build up a single matrix, which was subjected to a Principal Component Analysis (PCA) by means of the Statistica 6 programme (Statasoft Inc., USA).

3. Results

3.1. Grape berries

3.1.1. Levels of biogenic amines

Free biogenic amine levels were determined by HPLC in conventional, organic and biodynamic berries of Pignoletto and Sangiovese varieties (Fig. 1). In all the samples putrescine (PUT) was the most abundant polyamine followed by spermidine (SPD). Among monoamines, only tryptamine (TRYPT) was present and in both grape cultivars averaging 4.7-fold higher in red than in white berries (Fig. 1). The total level of amines in red berries was on average 5.5-fold higher than that of white berries independent of the agricultural method used, with respectively 2600 μmol/kgFW for conventional and biodynamic Sangiovese (SC and SB), 3100 μmol/kgFW for organic Sangiovese (SO) and 470, 360 and 670 μmol/kgFW respectively for conventional (PC), organic (PO) and biodynamic (PB) Pignoletto berries.

3.1.2. Levels of polyphenols

Total polyphenol (Fig. 2A) and anthocyanin (Fig. 2B and C) amounts were determined in Pignoletto and Sangiovese berries by using spectrophotometric methods. In particular total polyphenols resulted to be on average 4.7 and 5.3 g of gallic acid (GA) equivalents/kgFW (g GA eq/kgFW) respectively for Pignoletto and Sangiovese berries (Fig. 2A) with no significant difference among the three agricultural practices in both cultivars. As expected the levels of total anthocyanins were much higher in red than in white berries (about 100-fold for conventional and organic and 135-fold for biodynamic samples). In both Pignoletto and Sangiovese the samples deriving from conventional field management showed higher levels of anthocyanins followed by biodynamic and organic ones (Fig. 2B and C).

The detailed polyphenolic profile was determined by HPLC-DAD. Several compounds were detected, among which the most relevant were catechins (Fig. 2D) and stilbenes (Fig. 2E). In general total catechins were 13 to 46-fold higher than total stilbenes, with SB and SO having respectively the minimum and maximum difference (Fig. 2D and E). A different spectrum of catechins and stilbenes was detected in white and red grapes but not in berries of the same cultivar grown following different agricultural methods.

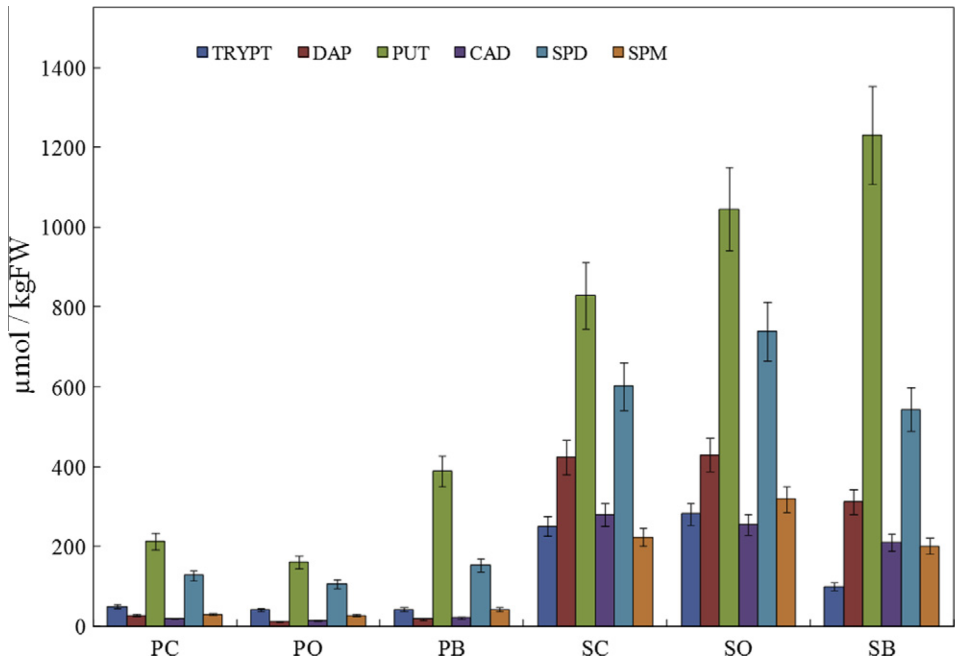

Fig. 1. Biogenic amine levels (µmol/kgFW) measured in Pignoletto and Sangiovese berries grown following conventional (PC and SC), organic (PO and SO) and biodynamic (PB and SB) agricultural practices. TRYPT, tryptamine; DAP, diamine-propane; PUT, putrescine; CAD, cadaverine; SPD, spermidine; SPM, spermine. Data are the mean ± SE ($n = 4$).

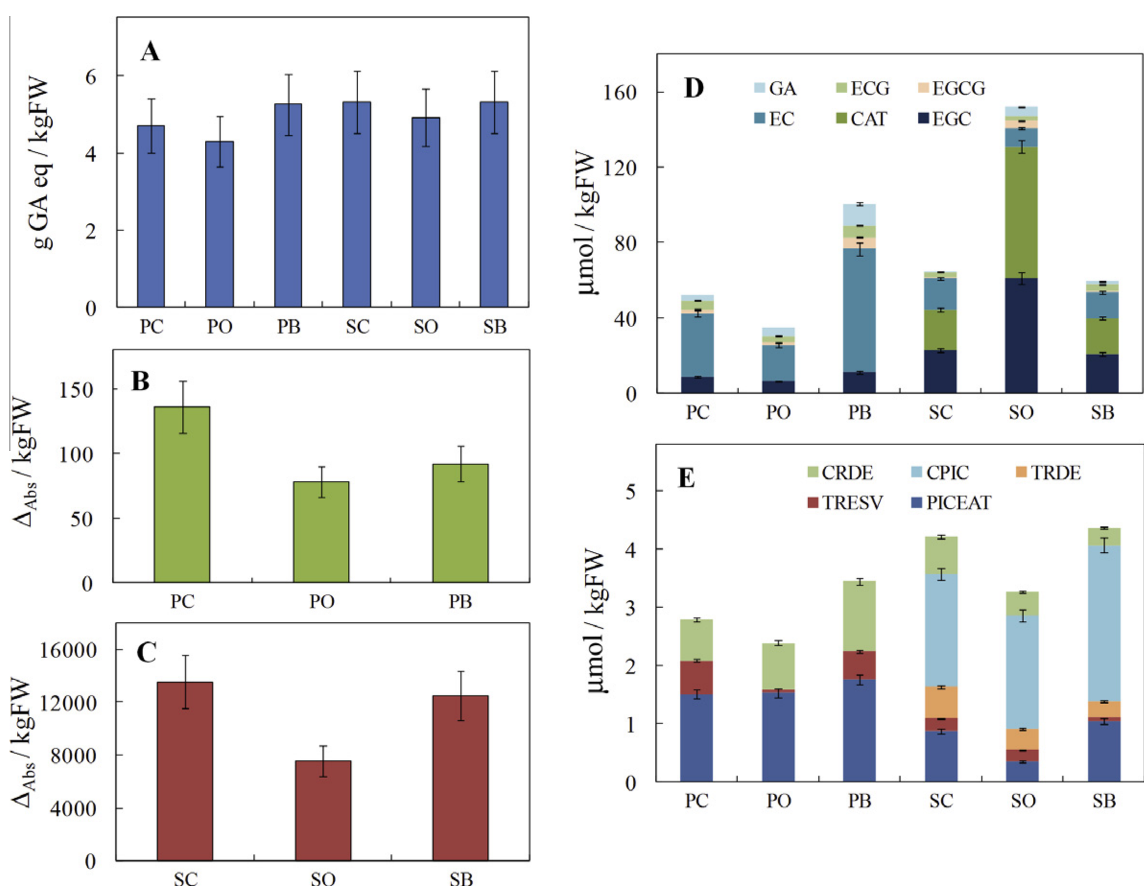

Fig. 2. Polyphenol levels in Pignoletto and Sangiovese berries grown following conventional (PC and SC), organic (PO and SO) and biodynamic (PB and SB) agricultural practices. (A) Total polyphenol quantification. Data are expressed as g of gallic acid (GA) equivalent per kilogram of fresh weight (g GA eq/kgFW). Data are the mean ± SE ($n = 4$). (B and C) Anthocyanin levels. Data are expressed as the variation of absorbance units for kilograms of fresh weight (Δ_{Abs}/kgFW). Data are the mean ± SE ($n = 4$). (D) Catechin levels (µmol/kgFW) measured by HPLC-DAD. GA, gallic acid; ECG, epicatechin-gallate; EGCG, epigallocatechin-gallate; EC, epicatechin; CAT, catechin; EGC, epigallocatechin. Data are the mean ± SE ($n = 4$). (E) Stilbene levels (µmol/kgFW) measured by HPLC-DAD. CRDE, *cis*-resveratroloside; CPIC, *cis*-piceid; TRDE, *trans*-resveratroloside TRESV, *trans*-resveratrol; PICEAT, piceatannol. Data are the mean ± SE ($n = 4$).

Epicatechin (EC) and catechin (CAT) were the catechins most abundant, respectively, in white and red berries (Fig. 2D). The highest levels of total catechins were detected in biodynamic for Pignoletto and in organic for Sangiovese berries. In general, the levels of stilbenes were slightly lower in Pignoletto than in Sangiovese grapes (Fig. 2E). Resveratrol (RESV) was detected both in the free (*trans*-RESV) and mono-glucosylated forms in all the samples. In particular *cis*-piceid (CPIC) and *trans*-resveratroloside (TRDE) were measured only in the red grapes (Fig. 2E). Piceatannol (PICEAT) was on average 2-fold higher in white than in red berries.

The levels of other four polyphenols were determined by HPLC-DAD (Table 1). In white berries only rutin (RUT) and vanillin (VAN) were detectable, while quercetin (QUERC) was present in trace amounts and myricetin (MYR) was absent. In red berries QUERC (average level of 7.1 μmol/kgFW) was the most abundant followed by VAN (average level of 0.6 μmol/kgFW) (Table 1). Naringenin and hydroxycinnamic acids were not detected either in Pignoletto or Sangiovese samples.

3.1.3. Antioxidant activity

The antioxidant activity of the different grape berries was measured by using the DPPH method. On average Pignoletto berries showed a 3-fold lower antioxidant capacity compared to Sangiovese ones (Table 2), with PB and SC having the highest activity respectively for white and red samples.

3.2. Wines

3.2.1. Levels of biogenic amines

The levels of biogenic amines were determined by HPLC in the wines, produced from Sangiovese and Pignoletto grapes grown under conventional, organic and biodynamic conditions, and following the respective oenological practices (Fig. 3). On average 3.6-fold higher levels of amines were detected in white wines (about 9500 μmol/L) in comparison to red ones, with the highest amount detected in PC. Red wines showed similar levels of biogenic amines (on average 2600 μmol/L) independent of the adopted winemaking methodology (Fig. 3). PUT and TRYPT were the most abundant amines respectively in white and red wines. In comparison to grape berries (Fig. 1), in wines two additional amines, histamine (HIM) and tyramine (TYM), were detected as a consequence of the microbial fermentation process that occurs during winemaking. HIM and TYM levels were on average respectively 3.8 and 5.7-fold higher in white than in red wines. TRYPT was present in similar amounts in all the analysed wines (Fig. 3), in contrast to grapes in which it was more abundant in red than in white berries (Fig. 1).

3.2.2. Levels of polyphenols

Total levels of polyphenols (Fig. 4A) and anthocyanins (Fig. 4B and C) were determined in conventional, organic and biodynamic wines obtained from Pignoletto and Sangiovese grapes. Total polyphenols were on average 6.5-fold higher in red than in white wines, with SC and PO showing respectively the highest values for red and white samples (Fig. 4A). Analogously to berries, the anthocyanin content of red wines was much higher than that of white wines (440, 62 and 217-fold respectively for conventional, organic and biodynamic samples), with the highest levels detected in PO and SB (Fig. 4B and C). The quantification of catechins by HPLC-DAD, evidenced a similar profile both in white and red wines (Fig. 4D). In particular, PO and SB showed the highest amount of total catechins, in agreement with the data on total polyphenols and anthocyanins (Fig. 4A–C). Red wines showed the highest levels of stilbenes with a wider spectrum of compounds compared to white wines (Fig. 4E). In particular, in white wines only free RESV was detected, both in *cis* (CRESV) and *trans* (TRESV) forms. In addition to free RESV, the three Sangiovese wines showed the presence of mono-glucosylated stilbenes (both RDE and PIC) and of PICEAT, with CPIC being the most abundant stilbene (Fig. 4E).

As previously shown for berries, the levels of four other polyphenols were determined by HPLC-DAD (Table 1). In white wines only QUERC and VAN were detectable, while RUT was present in trace amounts and MYR was absent. In Sangiovese wines, QUERC and RUT were the most abundant compounds with significantly higher levels in conventional wine, compared to organic and biodynamic wines (Table 1). In red wines, MYR and VAN were present at average levels of 219 and 318 μmol/L respectively. Naringenin and hydroxycinnamic acids were not detected in either Pignoletto or Sangiovese samples.

3.2.3. Antioxidant activity

The antioxidant activity measured by the DPPH method was on average 9.5-fold higher in red than in white wines (Table 2). This result seems in agreement with the levels of total polyphenols, anthocyanins and stilbenes reported (Fig. 4).

4. Discussion

The analytical data presented on white and red grapes and wines did not generally show a significant difference among the

Table 2
DPPH antioxidant activity of Pignoletto and Sangiovese berries and wines obtained following conventional (PC and SC), organic (PO and SO) and biodynamic (PB and SB) agricultural and oenological practices. Data are expressed as g of ascorbic acid (AA) equivalent per kilogram of fresh weight (g AA eq/kgFW) or per litre (g AA eq/L). Data are the mean ± SE ($n = 4$).

Samples	Berries (g AA eq/kgFW)	Wines (g AA eq/L)
PC	7.7 ± 0.3	0.5 ± 0.1
PO	7.2 ± 0.8	1.0 ± 0.1
PB	9.2 ± 1.3	0.8 ± 0.1
SC	31.3 ± 3.2	8.4 ± 0.1
SO	24.5 ± 0.6	6.8 ± 0.4
SB	26.4 ± 3.6	6.1 ± 0.5

Table 1
Content of additional polyphenols in Pignoletto and Sangiovese berries and related wines obtained following conventional (PC and SC), organic (PO and SO) and biodynamic (PB and SB) agricultural and oenological practices. The polyphenol levels (expressed as μmol/kgFW for berries and as mmol/L for wines) were measured by HPLC-DAD. QUERC, quercetin; RUT, rutin; MYR, mirycetin; VAN, vanillin. Data are the mean ± SE ($n = 4$).

Samples	Berries (μmol/kgFW)				Wines (μmol/L)			
	QUERC	RUT	MYR	VAN	QUERC	RUT	MYR	VAN
PC	Trace	0.76 ± 0.09	–	2.25 ± 0.56	82.22 ± 0.85	Trace	–	95.58 ± 5.99
PO	Trace	1.15 ± 0.34	–	1.88 ± 0.03	434.05 ± 4.77	Trace	–	129.43 ± 2.07
PB	Trace	0.46 ± 0.02	–	3.17 ± 0.61	212.33 ± 2.27	Trace	–	110.77 ± 2.25
SC	8.04 ± 0.87	0.22 ± 0.04	0.13 ± 0.02	0.70 ± 0.07	1700.13 ± 21.75	1893.10 ± 10.76	245.54 ± 0.12	158.83 ± 16.99
SO	5.99 ± 0.80	0.10 ± 0.01	0.26 ± 0.02	0.47 ± 0.03	903.41 ± 4.49	708.93 ± 2.17	222.38 ± 1.56	243.48 ± 1.64
SB	7.20 ± 0.83	0.10 ± 0.01	0.29 ± 0.04	0.63 ± 0.01	1470.12 ± 2.65	1039.94 ± 7.20	485.76 ± 3.85	254.25 ± 0.27

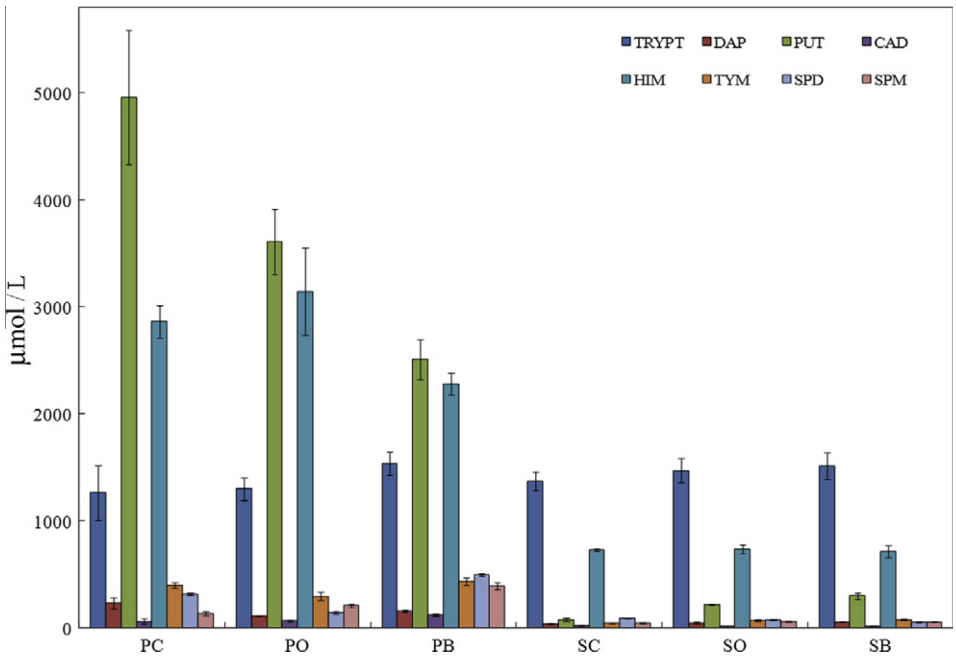

Fig. 3. Biogenic amine levels (µmol/L) measured in Pignoletto and Sangiovese wines obtained following conventional (PC and SC), organic (PO and SO) and biodynamic (PB and SB) oenological practices. TRYPT, tryptamine; DAP, diamine-propane; PUT, putrescine; CAD, cadaverine; HIM, histamine; TYM, tyramine; SPD, spermidine; SPM, spermine. Data are the mean ± SE ($n = 4$).

samples from different agricultural and winemaking practices (Figs. 1–4, Table 2), while, as expected, a greater difference was evident between white and red samples (in particular wines), in accordance with other published papers (Landete, Ferrer, Polo, & Pardo, 2005; Landrault et al., 2001; Minussi et al., 2003). Sangiovese berries showed the presence of higher amounts of biogenic amines (Fig. 1), of anthocyanins (Fig. 2B and C) and a higher antioxidant activity (Table 2), with respect to Pignoletto. In both white and red grapes the amine species are generally considered most dangerous for human health, namely HIM and TYM, were not detected, while high amounts of PUT were measured in red samples (Fig. 1). The HPLC-DAD analyses of catechins and stilbenes demonstrated a different spectrum of metabolites between white and red berries even though the total levels of these compounds are similar with the exception of PB and SO that showed higher levels of catechins (Fig. 2D and E).

In contrast to grape samples, the amount of biogenic amines was largely lower in red compared to white wines, which presented high amounts of HIM, TYM and PUT produced as a consequence of winemaking fermentation (Fig. 3). It has been demonstrated that HIM and TYM, but also other amines, may represent potential threats for human health and are mainly produced by bacteria of the *Lactobacillus* or *Oenococcus* genera which are usually present in the must during the fermentation process (Beneduce et al., 2010). The variability of the biogenic amine contents and/or profiles in wine could be explained on the basis of differences in the geographical region, grape variety, raw material quality, winemaking process, vintage, time and storage conditions and possible microbial contaminations (Beneduce et al., 2010). Therefore due to the large number of factors involved, it is not easily feasible, even though desirable, to minimise the formation of biogenic amines in wine making. The reduction of amine formation might be partially achievable by carefully selecting the grape variety and optimising the wine making parameters (such as temperature, maceration time, yeast strains). Cultivar related differences in biogenic amine content have been already observed for instance in Spanish (Landete et al., 2005), Greek (Soufleros, Buoloumpasi, Zotou, & Loukou, 2007) and Italian grapes and wines (Del Prete, Costantini, Cecchini, Morassut, & Garcia-Moruno, 2009) which include Sangiovese. No data have yet been published on Pignoletto grapes or wines.

Concerning our data, the higher levels of biogenic amines in Pignoletto compared to Sangiovese wines may be due to higher levels of amino acids generally present in white wines with respect to red ones. This difference was clearly demonstrated for Greek wines (Soufleros et al., 2007) in which a significantly higher average content of total amino acids in white wines, in comparison to rosé and red ones, was observed and correlated with higher total biogenic amine levels. In addition grape nitrogen fertilisation treatments (as those performed in conventional agricultural practices) can cause an increase of precursor amino acids (such as histidine, tyrosine and ornithine) and consequently of amine concentration in the must and finally in the wine (Beneduce et al., 2010; Soufleros et al., 2007). However, in the present study no significantly higher levels of amines were observed in conventionally grown grapes and related wines (both Pignoletto and Sangiovese) compared to organic and biodynamic ones, with the exception of higher PUT levels for conventional Pignoletto wine (Fig. 3).

Interestingly it has also been reported that high concentrations of some phenolic compounds (naturally present in red grapes) affect biogenic amine production by inhibiting lactic acid bacteria growth (Alberto, Arena, & Manca de Nadra, 2007). Therefore the low levels of biogenic amines in Sangiovese wines (Fig. 3) may also be due to the presence of large amounts of phenolic compounds (Fig. 4) that, by inhibiting the activity of naturally present bacteria, may reduce the formation of HIM, TYM and PUT, which by contrast are freely synthesised during Pignoletto fermentation (Fig. 3). In addition to low amine levels, red wines were also abundant in bioactive polyphenols, such as anthocyanins and stilbenes, and showed high antioxidant activity (Fig. 4 and Table 2). Interestingly, PICEAT and mono-glucosylated stilbenes (RDE and PIC) were only present in red wines, while in white ones only free RESV was detected (Fig. 4). The presence of mono-glucosylated stilbenes, that have been proven to possess antioxidant activity comparable to

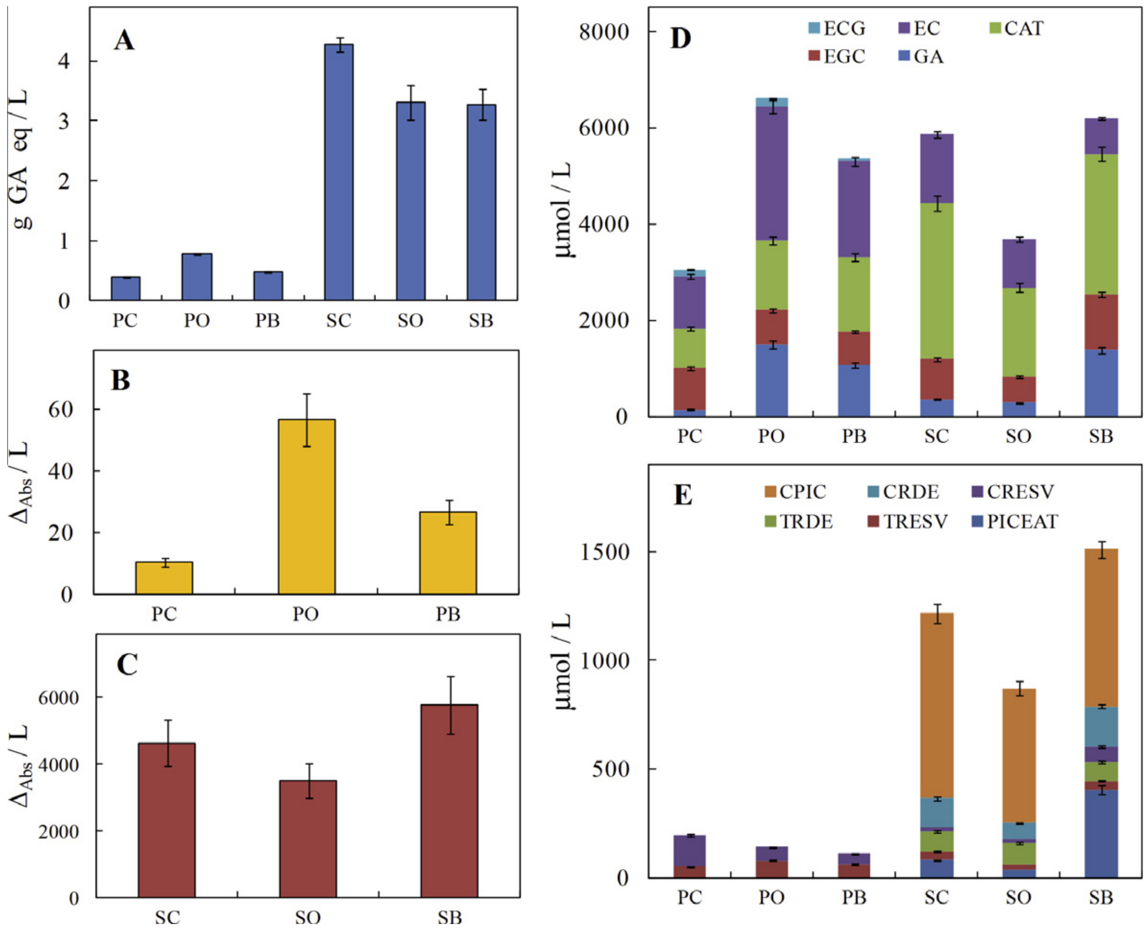

Fig. 4. Polyphenol levels in Pignoletto and Sangiovese wines obtained following conventional (PC and SC), organic (PO and SO) and biodynamic (PB and SB) oenological practices. (A) Total polyphenol quantification. Data are expressed as g of gallic acid (GA) equivalent per litre of wine (g GA eq/L). Data are the mean ± SE (n = 4). (B and C) Anthocyanin levels. Data are expressed as the variation of absorbance units for litre (Δ_{Abs}/L). Data are the mean ± SE (n = 4). (D) Catechin levels (µmol/L) measured by HPLC-DAD. GA, gallic acid; ECG, epicatechin-gallate; EC, epicatechin; CAT, catechin; EGC, epigallocatechin. Data are the mean ± SE (n = 4). (E) Stilbene levels (µmol/L) measured by HPLC-DAD. CPIC, *cis*-piceid; CRDE, *cis*-resveratroloside; CRESV, *cis*-resveratrol; TRDE, *trans*-resveratroloside TRESV, *trans*-resveratrol; PICEAT, piceatannol. Data are the mean ± SE (n = 4).

free RESV but with a more extended half-life and bioavailability (Regev-Shoshani et al., 2003), together with the lower amount of potentially toxic biogenic amines, seem to confirm the higher healthy characteristics of red compared to white wines. In this respect, the presence in red wines of PICEAT that was demonstrated to have an inhibitory activity on the PUT forming enzyme ornithine decarboxylase (ODC) (Wolter et al., 2004), was very interesting, confirming a possible beneficial effect of stilbenes. No significant differences were observed between the catechin profiles and the levels in white and red wines. In particular, EGCG, that was demonstrated to have an inhibitory activity both on the HIM forming enzyme, histidine decarboxylase (Nitta et al., 2007) and the PUT forming enzyme, ODC (Melgarejo et al., 2010), was not detected in both the white and red wines, while only low levels of this catechin were present in red grapes.

To better confirm that the three different agricultural and oenological practices did not directly influence the biochemical characteristics of grapes and wines, all the data on polyphenol and polyamine levels and on total antioxidant activity, were used to perform a Principal Component Analysis (PCA) (Fig. 5). As a result, grape and wine samples clearly separated from each other with white and red berries grouped together (Fig. 5A, group 1) and white and red wines clearly separated into two distinct groups (Fig. 5A, respectively group 2 and group 3). The PCA confirmed that, on the basis of the biochemical analyses performed, there was no difference between white or red grapes obtained from conventional, organic and biodynamic agricultural practices (Fig. 5A, group 1). A minimum difference was evidenced between red and white berries, which separated into two distinct subgroups within group 1 (Fig. 5A). The variables that mainly contributed to the grouping of grape samples were SPD, DAP, CAD, total anthocyanins (ANTH), antioxidant activity (ANTIOX) and EGCG (Fig. 5B). The PCA also evidenced a clear difference between white and red wines which was obviously derived from the different metabolic profile of grape variety and also by white and red winemaking practices, which determined the presence or absence of different types of metabolites and consequently the separation of wine samples into two distinct groups according to wine colour (Fig. 5A, groups 2 and 3). As also shown for grape samples, there was no difference among wines obtained from conventional, organic or biodynamic vinification practices. The variables that mainly contributed to the separation of wine samples into two distinct groups were PUT, ECG, TYM, HIM for white wines and PICEAT, TRDE, CRDE, CPIC, RUT and MYR for red wines (Fig. 5B) confirming previous detailed biochemical data (Figs. 3 and 4).

In conclusion it is well known that the interaction between different food metabolites (such as biogenic amines and polyphenols) and their relative biosynthetic pathways, may contribute to the healthy or detrimental characteristics of the food itself. Regarding Pignoletto and Sangiovese berries and wines (vintage 2009), our

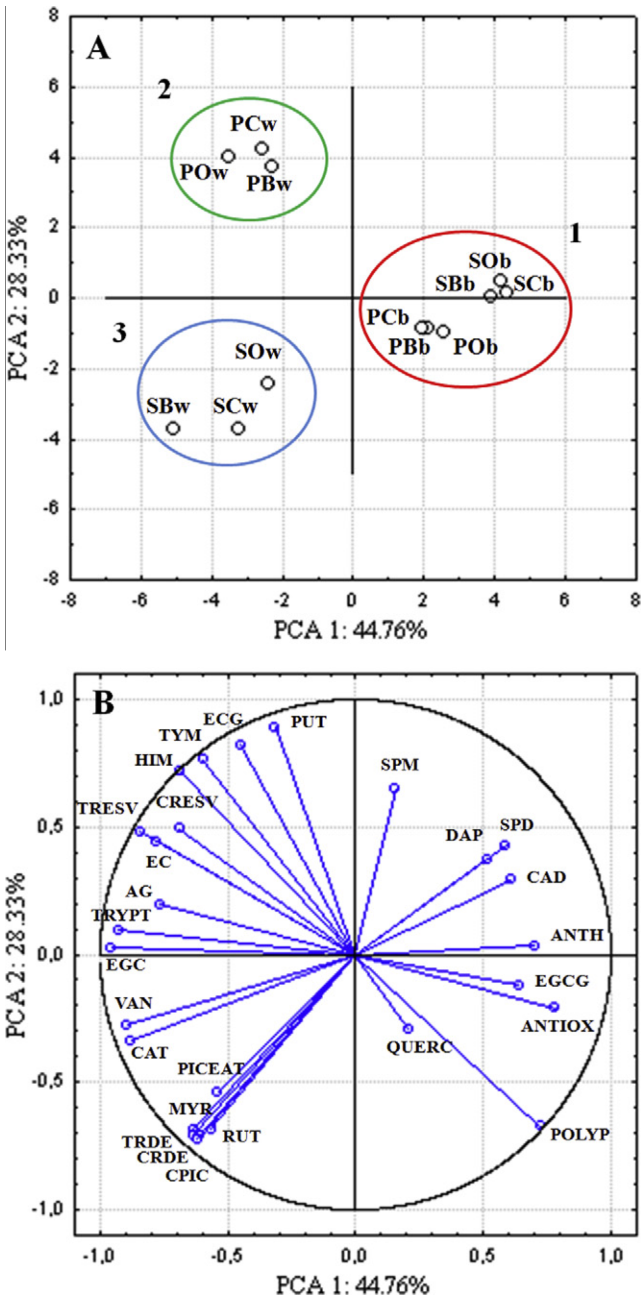

Fig. 5. Output of Principal Component Analysis (PCA) of the analytical data relative to Pignoletto and Sangiovese berries (PCb, POb, PBb, SCb, SOb, SBb) and wines (PCw, POw, PBw, SCw, SOw, SBw) obtained following conventional, organic and biodynamic practices. (A), PCA; (B) Contribution of the individual variables to the PCA. Total anthocyanins (ANTH); total polyphenols (POLYP); antioxidant activity (ANTIOX).

data seem to indicate that their metabolic profiles are not particularly influenced by the conventional, organic or biodynamic grape growth conditions or by the related wine making practices, but mainly by the varietal, physiological and metabolomic characteristics of the food raw material itself.

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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.2013.01.041.

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