The impact of mycorrhizal fungi on Sangiovese red wine production: Phenolic compounds and antioxidant properties

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ABSTRACT

The aim of this work was to analyze the effect of a microbiological consortium, in particular mycorrhizal fungi, on the phytochemical composition and on the antioxidant properties of Sangiovese wines with respect to wines from conventional agriculture, paying particular attention to their oxidative stability following oxygen exposure.

We determined by spectrophotometric methods and HPLC-DAD the phytochemical composition and by ORAC assay the antioxidant activity of wines. Besides, in order to evaluate the beneficial effects of both symbiotic and conventional wines, we investigated on human erythrocytes the cellular antioxidant activity (CAA) and the hemolysis inhibition.

Our results showed that symbiotic wines had both a better oxidative stability and a significantly higher level of bioactive compounds compared to the conventional ones. Despite the bioactive compounds variation, no difference in antioxidant capacity was found. However, erythrocytes pre-treated with symbiotic wines exhibited higher biological activities than the equivalent conventional one.

In conclusion, the use of a microbiological consortium represents an ecologically and economically relevant solution in vineyard cultivation to get high-quality wines, with improved nutritional and nutraceutical value.

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Keywords:
Phenolic composition
Antioxidant activity
Sangiovese wine
Mycorrhizal grapes
HPLC

1. Introduction

Wines produced using an environmentally sustainable approach, such as organic (Mann, Ferjani, & Reissig, 2012), biodynamic (Meunier, 2001; Parpinello, Rombolà, Simonì, & Versari, 2015; Preston, 2008; Zucca, Smith & Mitri, 2009), and biological practices (Alvarez-Sala, Slowing & Gomez-Serranillos, 2000) have recently enjoyed increasing popularity due to the growing demand for healthy products. The attention on soil treatments, their fertility and preservation is a very actual and discussed topic. It is well known, for instance, that sustainable practices improve the spore abundance and diversity of endophytic arbuscular mycorrhizal fungi (AMF), thus highlighting their greater ecological importance in the environment (Radic, Likar, Hančević, Bogdanovic, & Paskovic, 2014; Holland, Bowen, Bogdanoff, & Hart, 2014). The AMF colonize the roots of several land species and, in exchange of fixed carbon compounds, provide the host plant with soil mineral nutrients, mainly P, N, Ca, Fe, Cu, and Zn. Besides, the AMF increase biotic (root pathogens) and abiotic (drought, salinity, heavy metals) stresses resistance of the host, thus promoting plant growth and productivity (Balestrini, Magurno, Walker, Lumini, & Bianciotto, 2010; Sikes, 2010; Giovannetti et al., 2012; Holland et al., 2014; Radic et al., 2014). These fungal symbionts are receiving much attention in vineyard production systems. Indeed, grapevines are positively affected by AMF symbiosis especially in terms of plant growth and development, biomass production, drought tolerance, as well water, mineral, and nutrient uptake (Balestrini et al., 2010; Likar, Hančević, Radič, & Regvar, 2013; Holland et al., 2014; Radic et al., 2014). The most common mycorrhizal species associated to vine roots belong to Glomus genus (Balestrini et al., 2010; Schreiner & Mihara, 2009). These endomycorrhizal fungi penetrate the cortex cells of vine roots forming arbuscular and vesicular structures and develop an intensive network of long and stringy hyphae, which increase the contact surface between the vine roots and the surrounding soil (Schreiner, 2005). Besides, plants take advantage from endomycorrhizal symbiosis thanks to the small diameters of fungal mycelia (2–4 μm), which enable the micro-sized cross section of hyphae to penetrate and colonize large soil portions otherwise unreached by coarse roots (Maseko & Dakora, 2013). Furthermore, mycorrhizal fungi improve nutrients absorption secreting enzymes able to metabolize compounds generally unavailable to the host plant (Kaur, Singh, & Kang, 2014).

Several studies have investigated the arbuscular mycorrhizal fungi effect on vines (Vitis spp.) growing (Camprubí et al., 2008) and soil fertility in different soil types and vineyard age (Schreiner & Mihara,
2009), paying particular attention on fungal population and biodiversity, as well on quality of soil in organic versus conventional vineyards (Radic et al., 2012, 2014; Riches et al., 2013; Freitas, Yano-Melo, da Silva, de Melo & Maia et al., 2011; Schreiner & Mihara, 2009; Smith et al., 2008). However, few studies deal with the comparison among wines produced by symbiotic or conventional approaches, in terms of quality, sensory properties, and beneficial effects on human health. This last point is strictly related to the content of minor components, which can be highly influenced by climatic conditions, “terroir”, grapevine types, winemaking processes, age, and year of vintage (Lachan, Sulc, & Faitova, 2009). Among the most important classes of minor components, polyphenols (Rodriguez-Delgado, Gonzales-Hernandez, Conde-Gonzales, & Perez-Trujillo, 2002), flavonols, flavonoids, and anthocyanins (Tsanova-Savova & Ribarova, 2002) contribute to the antioxidative activity of red wine (Chedea, Braicu, & Socaciu, 2010; Procházková, Boušová, & Wilhelmová, 2011; Romani, Mancini, Tatti, & Vincieri, 1996).

So, to make a solid comparison among symbiotic and conventional wines, we analyzed red wine samples, supplied by the Tenuta Rubbia al Colle in Tuscany (Arcipelago Muratori Company, Italy), got from Sangiovese grapevines (Vitis vinifera L) grown in similar climatic conditions and following the same winemaking procedure. These are very important starting conditions to state that all these wine samples only differ in the “soil treatment”. In particular, we tested on Sangiovese grapevines the effect of the Micosat F inoculum, a microbiological consortium of agronomically useful soil microorganisms comprising spores and mycelia of endomycorrhizal fungi of Glomus genus and, to a lesser extent, of rhizosphere bacteria, saprophytic fungi and yeast.

Thus, the aim of this work was to investigate the benefits of this microbiological consortium on phytochemical composition and antioxidative activity of symbiotic wines compared to ones from conventional agriculture, paying particular attention to the oxidative stability of wines following oxygen exposure.

Therefore, we have compared symbiotic and conventional red wine samples exposed to different level of oxygen. We determined the phytochemical composition, specifically total polyphenols, flavonoids, flavonols, and anthocyanins, by spectrophotometric methods. Then, we analyzed eight analytes (gallic acid, 3,4 DHB acid, tyrosol, resveratrol, caffeeic acid, quercetin, malvidin(chloride) and isorhamnetin) by HPLC-DAD according to the method proposed by Iberni-Gómez, Andrés-Lacueva, Lamuela-Raventós, and Waterhouse (2002). Moreover, we evaluated the potential beneficial effects of symbiotic and conventional red wines on human erythrocytes as cellular antioxidant activity (CAA) and oxidative hemolysis inhibition.

2. Materials and methods

2.1. Chemicals and reagents

All standards and reagents were of analytical grade. Methanol, acetonitrile, trifluoracetic acid, sodium carbonate, sodium hydroxide, potassium chloride, sodium acetate, Folin-Ciocalteu reagent, catechin hydrolysis, gallic acid, quercetin dihydride, resveratrol, malvidin(chloride), vanillic acid, caffeic acid, riboflavin, 3,4 DHB acid, tyrosol, isorhamnetin, tryptophan, 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), 2′-a-asozob (2-amidinopropane) dichlororhodine (AAPH), fluorescein sodium salt, and 2′,7′-dichlororfluorescein diacetate (DCFH-DA) were purchased from Fluka-Sigma-Aldrich, Inc. (St. Louis, MO). Sodium nitrite and aluminum chloride were purchased from Carlo Erba (Milan, IT), phosphate buffer saline (PBS) tablet and absolute ethanol were purchased from WWR (Radnor, PA), while hydrochloric acid was purchased from Merck (Readington, NJ).

2.2. Wine samples

Different types of red wine made with conventional or mycorrhizal Sangiovese grape varieties (V. vinifera L.) were supplied by Tenuta Rubbia al Colle in Tuscany (Arcipelago Muratori Company, Italy).

The mycorrhizal Sangiovese vineyard was treated with different MICOSAT F preparations exerting bio-stimulating action on soil and vines. In particular, these preparations consist in a crude inoculum of shedded mycorrhizal roots containing spores and hyphae of endomycorrhizal fungi of Glomus genus (e.g. G. mosseae GP 11, G. viscosum GC 41, G. intraradices GB 67 and GG 31, G. coronatum GU 53, G. caledonian GM 24) and Rhizophagus (Rhizophagus irregularis RI 31). Moreover, it contains other active microorganisms, to a less extent, such as: rhizosphere bacteria (e.g. Agrobacterium radiobacter AR 39, Pseudomonas spp PN 01; Pseudomonas fluorescens PA 28, Bacillus subtilis BA 41 and BR 62, Streptomyces spp. SB 14 and ST 60), saprophytic fungi (e.g. Trichoderma harzianum TH 01, Tricho-
derma viridae TV 03, Pochonia chlamydosporia PC 50, Ulocladium oudemansii UO 18) and yeast (Pichia pastoris PP 59).

Specifically, we compared three conventional (CON) and symbiotic (SMH) Sangiovese red wines, both exposed to 0, 24, and 48 mg L⁻¹ of oxygen corresponding to 0 (T002), 3 (T102) and 6 (T202) porings. All wine bottles were stored at 25 °C and away from sources of light and heat. At the opening of each bottle, an analysis sample of 250 ml was created in a dark glass Duran bottle with screw cap certified UNI EN ISO 4796-1:2001. These samples were stored at 4 °C. The analyzes were carried out within 24 h of opening.

2.3. Phytochemical characterization

Total phenolic compounds were determined by the Folin-Ciocal-
tee colorimetric method (Singleton, Orthofer, & Lamuela-Raventos, 1999) and expressed as mg of gallic acid equivalents (GAE)/L. Total flavonoids were quantified using the aluminum chloride colori-
metric method (Kim, Chun, Kim, Moon & Lee, 2003) and expressed as mg catechin equivalents (CE)/L. Total flavonoids were measured according to the method described by Romani et al. (1996) and expressed as mg quercetin equivalents (QE)/L. Total monomeric anthocyanins were determined using the pH differential method (Lee, Durst, & Wrolstad, 2005) and expressed as mg cyanidin-3-glucoside equivalents (C3G)/L (cyd-gl, molar extinction coefficient of 26,900 L/(cm mol) and molecular weight of 449.2 g/mol).

2.4. Oxygen radical absorbance capacity (ORAC) assay

The antioxidant capacity of red wine samples was quantified using the oxygen radical absorbance capacity (ORAC) assay as described by Gabriele et al. (2015). AAPH was used as peroxyl radicals generator and fluorescein as a probe. Fluorescein fluorescence decay was read at 485 nm excitation and 514 nm emission using a VictorTM X3 Multilabel Plate Reader (Waltham, MA) and Trolox was used as a standard. ORAC values were expressed as millimoles per liter of Trolox equivalents (mmol TE/L).
2.5. HPLC method

The chromatographic measurements were performed using a JASCO HPLC system, equipped with interface LC-Net II/ADC, autosampler AS-950 with a rotary valve injector and a loop of 100 μL, a low pressure quaternary gradient pump PU-2089 Plus with eluent degasser and spectrophotometric diode array detector MD-2010 Plus. HPLC chromatograms were analyzed by the ChromNAV (Chromatography Data System) software. An Agilent Technologies TC-C18 column (150 mm × 4.6 mm, 5 μm PS) equipped with a C18 guard column (Agilent Technologies) was used. All samples were filtered on a Whatman 4 mm dedicated filter with a poly(1,1,2,2-tetrafluoroethyline) (PTFE) 0.45 μm membrane. Then, 0.5 mL of the filtered solution were diluted (1:10) with bi-distilled water and 50 μL of the got sample were injected into the column.

To identify and quantify the analytes of interest, we followed the method proposed by Ibern-Gómez et al. (2002). The quantification of analytes was performed by the external standard calibration method, whose details and validation are reported in the Supporting Information.

2.6. Preparation of erythrocytes

Human blood samples from healthy volunteers were collected in ethylenediaminetetraacetic acid (EDTA)-treated tubes and centrifuged for 10 min at 2300 × g at 4 °C. Plasma and buffy coat were discarded and erythrocytes were washed twice with PBS pH 7.4.

2.7. Cellular antioxidant activity (CAA) assay in red blood cells

The antioxidant activity of red wine samples was evaluated in an *ex vivo* erythrocytes system as described by Blasa, Angelino, Gennari, and Ninfali (2011). The fluorescence was read at 485 nm excitation and 535 nm emission by using a Victor™ X3 Multilabel Plate Reader (Waltham, MA). Each value was expressed as follows: CAA unit = 100−(∫SA/∫CA) × 100, where ∫SA is the integrated area of the sample curve and ∫CA is the integrated area of the control curve (Wolfe & Liu, 2007).

2.8. Erythrocytes oxidative hemolysis

The erythrocytes hemolysis was measured according to the method described by Mikstacka, Rimando, and Ignatowicz (2010) and the oxidative stress was generated by thermal decomposition of AAP in peroxyl radicals. The erythrocytes oxidative hemolysis was spectrophotometrically evaluated at 540 nm as hemoglobin released in the supernatant. Control and blank samples were used and refer to erythrocytes exposed to AAPH or PBS, respectively. Each value was expressed as a percentage of hemolysis with respect to control.

2.9. Data analysis

The data analysis was performed using GraphPad Prism, version 4.00 for Windows (GraphPad software, San Diego, CA). Assays were carried out in triplicate and the results were expressed as mean values ± standard deviation (SD). Differences among the conventional wines and the symbiotic ones were analyzed by one-way analysis of variance (ANOVA) with Dunnett’s multiple comparison test. An unpaired *t*-test was used to compare each conventional wine sample with the corresponding symbiotic one. The HPLC-DAD data were analysed by pooled *t*-test at 95% confidence level. All statistics were performed with significance at *p*-value lower than 0.05.

3. Results

3.1. Phytochemical profile and antioxidant capacity of red wine samples

Both conventional and symbiotic wine samples were screened for total polyphenol, flavonoid, flavanol, and anthocyanin content. As shown in Table 1, our results demonstrated that unexposed symbiotic wines showed significantly higher levels of flavonoids compared to the conventional one (**p < 0.01 vs CON T0O1**).

Besides, oxygen exposure decreased in a dependent dose-manner the conventional wine phenolic content, with a significant reduction in the conventional wine exposed to the highest levels of oxygen dose with respect to the unexposed one (**p < 0.05, CON T2O2 vs CON T0O1**). Otherwise, both symbiotic oxygen-exposed wines (SMB T1O2 and SMB T2O2) exerted a better oxidative stability of bioactive compounds with significantly higher levels of polyphenols (**p < 0.001 vs CON T2O2), flavonoids (**p < 0.01 vs CON T1O2; **p < 0.01 vs CON T0O1**) and flavanols (**p < 0.01 vs CON T1O2; **p < 0.01 vs CON T0O1**) than the equivalent exposed conventional ones. No difference in phytochemical composition was observed among symbiotic wine samples.

However, a significant reduction of total anthocyanins compared to the conventional wines was found (**p < 0.001 vs CON T0O1; **p < 0.01 vs CON T1O2; **p < 0.001 vs CON T2O2**).

The antioxidant capacity was evaluated by the ORAC assay in both conventional and symbiotic wines and the results are listed in Table 2. Despite the bioactive compounds variation, no difference among the ORAC values was observed.

<table>
<thead>
<tr>
<th>Phenolic Compounds</th>
<th>CON T0O1</th>
<th>CON T1O2</th>
<th>CON T2O2</th>
<th>SMB T0O2</th>
<th>SMB T1O2</th>
<th>SMB T2O2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polyphenols (mg GAE/L)</td>
<td>1089.2 ± 59.2</td>
<td>982.4 ± 93.9</td>
<td>881.1 ± 16.7*</td>
<td>1094.8 ± 59.2</td>
<td>1058.3 ± 35.8</td>
<td>1128.6 ± 35.1***</td>
</tr>
<tr>
<td>Flavonoids (mg CE/L)</td>
<td>437.6 ± 19.3</td>
<td>426.8 ± 6.6</td>
<td>431.4 ± 13.1</td>
<td>471.7 ± 14.2</td>
<td>474.8 ± 5.4***</td>
<td>508.9 ± 23.3**</td>
</tr>
<tr>
<td>Flavonols (mg C3G/E/L)</td>
<td>127.7 ± 7.1</td>
<td>133.4 ± 11.6</td>
<td>129 ± 0.001</td>
<td>183 ± 8.9***</td>
<td>184.9 ± 11.6**</td>
<td>182.4 ± 18.6**</td>
</tr>
<tr>
<td>Anthocyanins (mg C3G/E/L)</td>
<td>75.7 ± 5.1</td>
<td>75.7 ± 3.9</td>
<td>81.3 ± 1.9</td>
<td>49 ± 1.9***</td>
<td>47.9 ± 7.7***</td>
<td>46.8 ± 0.03***</td>
</tr>
<tr>
<td>ORAC (mmol TE/L)</td>
<td>35.5 ± 0.9</td>
<td>36 ± 0.6</td>
<td>35.9 ± 0.6</td>
<td>36.3 ± 0.7</td>
<td>36.1 ± 2.2</td>
<td>35.7 ± 1.6</td>
</tr>
</tbody>
</table>
3.2. HPLC-DAD analysis of wine samples

The chromatographic separation method used in this work has been modified starting from the method developed by Ibern-Gómez et al. (2002). In our case, the eluents were the same, but the column was different. For this reason, the elution gradient was optimized to adapt it to the column in use, as described in the Supporting Information. This method provided to be robust, with a good precision and high reproducibility. The percent variation coefficient (CV%) ranges between 0.1 and 1.9 for the investigated compounds. Details on the validation method (R² of the calibration curves, LOD, and LOQ) are reported in the Supporting Information.

For each wavelength of observation, in Fig. 1 we compared the chromatograms of a standard solution containing the analytes of interest (in red) and the SMB T0O2 ones (in black). The chromatograms of the wine samples are characterized by a broad baseline, due to the presence of many other components, that, however, did not affect the identification and quantification of the peaks of interest. The concentration of the different analytes was determined for all investigated samples and the results are summarized in Table 2.

These results show that the unexposed symbiotic wine sample contains higher concentrations of all analytes. This is particularly evident for gallic acid and 3,4 DHB acid (SMB T0O2 contains about 40% gallic acid and 300% 3,4 DHB acid more than CON T0O2). Otherwise, this effect is less pronounced, but still evident, for all other flavonoids and anthocyanins (SMB T0O2 contains about 30% tyrosol, 25% resveratrol, 12% caffeic acid, 23% quercetin, 5% isorhamnetin, and 37% malvidin chloride more than CON T0O2). The exposure to increasing oxygen concentrations produces a decrease of the analytes.

![Fig. 1](image_url)

**Table 2**

Values were expressed as mean (in µg/g) of three replicates; the confidence interval (CI) is expressed as \( \pm ts/\sqrt{N} \) at 95% confidence level and SD are reported in µg/g.

<table>
<thead>
<tr>
<th>Analytes</th>
<th>CON T0O2</th>
<th>CON T1O2</th>
<th>CON T2O2</th>
<th>SMB T0O2</th>
<th>SMB T1O2</th>
<th>SMB T2O2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CI</td>
<td>SD</td>
<td>CI</td>
<td>SD</td>
<td>CI</td>
<td>SD</td>
</tr>
<tr>
<td>Gallic acid (µg/g)</td>
<td>2.301 ± 0.040</td>
<td>0.016</td>
<td>2.180 ± 0.027</td>
<td>0.011</td>
<td>2.130 ± 0.027</td>
<td>0.011</td>
</tr>
<tr>
<td>3,4-DHB acid (µg/g)</td>
<td>0.090 ± 0.005</td>
<td>0.002</td>
<td>0.085 ± 0.005</td>
<td>0.002</td>
<td>0.082 ± 0.005</td>
<td>0.002</td>
</tr>
<tr>
<td>Tyrosol (µg/g)</td>
<td>2.029 ± 0.12</td>
<td>0.01</td>
<td>1.230 ± 0.017</td>
<td>0.007</td>
<td>&lt;LOQ</td>
<td>0.007</td>
</tr>
<tr>
<td>Resveratrol (µg/g)</td>
<td>0.092 ± 0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.122 ± 0.000</td>
</tr>
<tr>
<td>Caffeic acid (µg/g)</td>
<td>1.230 ± 0.004</td>
<td>1.005 ± 0.007</td>
<td>0.003</td>
<td>0.095 ± 0.002</td>
<td>0.001</td>
<td>1.395 ± 0.10</td>
</tr>
<tr>
<td>Quercetin (µg/g)</td>
<td>1.130 ± 0.019</td>
<td>0.872 ± 0.037</td>
<td>0.015</td>
<td>0.530 ± 0.022</td>
<td>0.009</td>
<td>1.461 ± 0.060</td>
</tr>
<tr>
<td>Isorhamnetin (µg/g)</td>
<td>0.220 ± 0.002</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.231 ± 0.005</td>
</tr>
<tr>
<td>Malvidin chloride (µg/g)</td>
<td>0.050 ± 0.002</td>
<td>0.001</td>
<td>0.045 ± 0.002</td>
<td>0.001</td>
<td>0.040 ± 0.002</td>
<td>0.001</td>
</tr>
</tbody>
</table>

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For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.
content, as expected. However, our data, analysed by pooled t-test at 95% confidence level, show that the symbiotic wines contain a higher concentration of analytes than the conventional ones. As shown in Table 2, all values are above the LOQ (except for tyrosol concentration for one sample, CON T2O2). Interestingly, resveratrol and isorhamnetin disappear in conventional wines after oxygen exposure, while they are still detectable in the symbiotic wines (SMB T1O2 and SMB T2O2). Moreover, as reported in the previous Section 3.1, both symbiotic oxygen-exposed wines (SMB T1O and SMB T2O2) have a better oxidative stability of all bioactive compounds than the equivalent exposed conventional ones. The effect of the second oxygen pouring in decreasing the concentration of the analytes (CON T2O2 versus CON T1O2 and SMB T2O2 versus SMB T1O2) is much less pronounced in symbiotic wines than in conventional wines.

3.3. Red wine cellular antioxidant activity (CAA) in red blood cells

The antioxidant activity of conventional and symbiotic red wine samples was evaluated using a cell-based model of human erythrocytes exposed to a peroxyl radical generator, the AAPH. Each value was expressed as CAA unit according to the Wolfe and Liu (2007) formula.

As shown in Fig. 2, pre-treated erythrocytes with unexposed symbiotic wine exhibited significantly higher CAA values than the conventional one (p < 0.05, SMB T0O2 vs CON T0O2); besides, both symbiotic oxygen-exposed wines have significantly raised the erythrocytes antioxidant activity compared to the conventional ones (p < 0.05 vs CON T1O2, **p < 0.05 CON T2O2). Furthermore, all conventional and symbiotic wines, as well as quercetin, exerted a significant increase of CAA values compared to control, referring to AAPH-treated erythrocytes (**p < 0.001 vs AAPH, CAA unit = 0). However, no difference in cellular antioxidant activity was observed among conventional or symbiotic pre-treated erythrocytes samples.

3.4. Red wine anti-hemolytic activity

The anti-hemolytic activity of conventional and symbiotic red wines was screened in comparison to erythrocytes exposed to AAPH, causing a strong oxidative hemolysis. As shown in Fig. 3, all wine pre-treatments exerted a significant hemolysis inhibition compared to the AAPH-treated erythrocytes (AAPH).

Specifically, we found a slightly higher anti-hemolytic effect in SMB T0O2 pre-treated erythrocytes than in the equivalent conventional ones. Besides, the symbiotic wines exposed to the highest oxygen doses exhibited a significantly higher hemolysis inhibition than the equivalent conventional one (**p < 0.01, SMB T2O2 vs CON T2O2). Furthermore, a significantly higher anti-hemolytic effect was observed in the CON T0O2 pre-treated erythrocytes with respect to the conventional wine exposed to the highest oxygen concentrations (p < 0.05 vs CON T0O2). Lastly, we determined the best hemolysis inhibition in symbiotic wine samples in comparison to the conventional ones.

4. Discussion

The present study evaluated the effect of a bio-friendly approach on the quality of wines undergoing to the same winemaking process. Specifically, we investigated whether the use of a microbiological consortium, in particular microcortial fungi, can contribute to getting high-quality wines with improved nutritional and nutraceutical value.

Despite of several studies have been published (Freitas, Yano-Melo, da Silva, de Melo, & Maia, 2011; Radic et al., 2012, 2014; Riches et al., 2013; Schreiner & Mihara, 2009; Smith et al., 2008) dealing with quality of soil, fungal population and diversity, in organic versus conventional vineyards, data on antioxidant properties and biological effects of conventionally and organically produced wines are missing. Indeed, only few studies have compared the phenolic composition and the antioxidant activity of wines got from organic and conventional productions (Vinkovic Vreck, Bojic, Zuntar, Mendas & Medić-Saric, 2011; Mulero, Pardo, & Zafrilla, 2010). None data on wines from microcortial grape varieties are known.

Thus, this work investigated whether the use of a microbiological consortium could positively affect the phytochemical composition and the antioxidant properties of Sangiovese wines with respect to wines from conventional agricultural productions, paying particular attention to their oxidative stability following oxygen exposure. Besides, for the first time we have analyzed the biological effects of

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**Fig. 2.** Cellular antioxidant activity (CAA) in human erythrocytes of conventional (CON) and symbiotic (SMB) wine samples exposed to 0, 24 and 48 mg L⁻¹ of oxygen, corresponding to 0 (T0O2), 3 (T1O2) and 6 (T2O2) pourings. Quercetin was used as reference standard. Values were expressed as mean ± SD of three determinations. Unpaired t-test: ¹ vs CON T0O2; ² vs CON T1O2; ³ vs CON T2O2.

**Fig. 3.** Anti-hemolytic activity of conventional (CON) and symbiotic (SMB) wine samples exposed to 0, 24 and 48 mg L⁻¹ of oxygen, corresponding to 0 (T0O2), 3 (T1O2) and 6 (T2O2) pourings. Trolox was used as reference standard. Values were expressed as mean ± SD of three determinations. One way ANOVA with Dunn’s post-hoc test: ¹ vs AAPH; ² vs CON T0O2. Unpaired t-test: ³ vs CON T0O2; ² vs CON T1O2; ³ vs CON T2O2.
both conventional and symbiotic wines on an in vitro cell model system of human erythrocytes to evaluate the effects of wine pre-treatment on the cellular antioxidant activity and on the hemolysis inhibition.

In order to evaluate the bioactive compounds content we used a chromatographic separation method, based on HPLC-DAD, adapting the method developed by Ibern-Gómez et al. (2002) for red wine. In particular, we have quantified the following eight phenolic compounds (benzoic acids, flavan-3-ols, cinnamic acids, flavonols and anthocyanins): gallic acid, 3,4 DHB acid, tyrosol, resveratrol, caffeic acid, quercetin, isorhamnetin and malvidin(chloride). The method is rather robust, with high reproducibility, excellent precision, and a percent variation coefficient (CV%) less than 2% for all analytes.

Interestingly, for all eight analytes, the concentration obtained in symbiotic wines is higher than in conventional wines. In all case, the obtained values have a high level of confidence (t-test at 95%). These results agree with those described by Vinkovic Vreck et al. (2011) for Croatian organic wines where they found a higher phytochemicals concentration, in particular chlorogenic acid, ferulic acid, catechin, trans-resveratrol, hydroxybenzoic acid and flavonols, and a higher radical scavenging activity than the conventional wines. Otherwise, Mulero et al. (2010) in organic wine from the Monastrell grape variety found a slightly higher level of phenolic compounds and antioxidant activity than the conventional ones.

When the wine is exposed to the lowest oxygen concentration, all analytes decrease both in conventional and symbiotic wines, even though the decreasing is different depending on the polyphenol. Conversely, the effect of the highest oxygen exposure is less critical than the lowest one in both types of wines.

As a general result, based on the bioactive compounds concentration determined by spectrophotometric methods and HPLC-DAD, symbiotic wines show a better oxidative stability with respect to the conventional ones. However, significantly lower levels of monomeric anthocyanins were detected in symbiotic wines than in conventional ones. On the contrary, a higher level of malvidin(chloride) was found in symbiotic wines (range 0.062–0.080 µg/g) compared to the conventional ones (range 0.040–0.050 µg/g).

During wine storage, monomeric or free anthocyanins are incorporated into polymeric pigments; thus, the observed anthocyanins reduction could be due to macromolecular complex formation, excluded by spectrophotometric analysis.

Despite the bioactive compounds variation, no difference in antioxidant capacity was found. However, symbiotic wines exerted a better biological activity in human erythrocytes with significantly increased value of cellular antioxidant activity and a better anti-hemolytic effect than the conventional ones.

5. Conclusions

In this study, we investigated whether the use of a microbiological consortium is able to positively affect the quality of Sangiovese wines under oxidative conditions. In summary, our results demonstrated that symbiotic wines showed both a better oxidative stability and a significantly higher level of bioactive compounds compared to the conventional wines. Furthermore, erythrocytes pre-treated with symbiotic wines exhibited higher biological activities than the conventional ones. Thus, the use of microbiological consortium in vineyards cultivation represents an ecologically and economically relevant solution in the sustainable wine productions. Indeed, it gives huge benefits for the farmers who can obtain high-quality products with an improved nutritional and nutraceutical content, reducing concurrently the use of chemical treatments, an important matter for both consumers and producers.

Conflict of interest

The authors declare no competing financial interest.

Acknowledgments

The authors wish to express their thanks to the Tenuta Rubbia al Colle in Tuscany (Arcipelago Muratori Company, Italy) for providing wine samples.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.lwt.2016.04.044.

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