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# Polyphenols content, phenolics profile and antioxidant activity of organic red wines produced without sulfur dioxide/sulfites addition in comparison to conventional red wines

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ABSTRACT

Wine exerts beneficial effects on human health when it is drunk with moderation. Nevertheless, wine may also contain components negatively affecting human health. Among these, sulfites may induce adverse effects after ingestion. We examined total polyphenols and flavonoids content, phenolics profile and antioxidant activity of eight organic red wines produced without sulfur dioxide/sulfites addition in comparison to those of eight conventional red wines. Polyphenols and flavonoids content were slightly higher in organic wines in respect to conventional wines, however differences did not reach statistical significance. The phenolic acids profile was quite similar in both groups of wines. Antioxidant activity was higher in organic wines compared to conventional wines, although differences were not statistically significant. Our results indicate that organic red wines produced without sulfur dioxide/sulfites addition are comparable to conventional red wines with regard to the total polyphenols and flavonoids content, the phenolics profile and the antioxidant activity.



## 1. Introduction

Oxidative stress is involved in the pathology of many diseases, such as atherosclerosis, diabetes, neurodegenerative diseases, aging and cancer (Aruoma, 1998). Dietary antioxidants may afford protection against oxidative stress-related diseases. Among dietary antioxidants, phenolics compounds are by far the most abundant in most diets (Aruoma, 1998; Block, Patterson, & Subar, 1992). Epidemiological studies strongly suggest that long-term consumption of polyphenols-rich foods offers some protection against the development of cancer, cardiovascular diseases, diabetes, osteoporosis and degenerative diseases (Aruoma, 1998). For individuals regularly consuming wine, coffee, beer and tea, these beverages will likely be the major sources of phenolics. It has been recognized that wine can have beneficial effects on human health when drunk in moderation. Epidemiological studies from numerous different populations show that individuals with the habit of moderate daily wine consumption present significant reduction in all-cause and particularly cardiovascular mortality when compared to abstainers or those who drink excess alcohol (Guilford & Pezzuto, 2011; Hertog, Feskens, Hollman, Katan, & Kornhout, 1993; Renaud & de Lorgeril, 1992). Human intervention studies investigating moderate levels of consumption also support the health benefits of wine. Hypolipidemic, hypotensive and anti-atherosclerotic effects, antioxidant status improvement and reduction in oxidation biomarkers have been described (Cooper, Chopra, & Thurnham, 2004; Cordova, Jackson, Berke-Schlessel, & Sumpio, 2005; Perez-Jimenez & Saura-Calixto, 2008).

The total amount of polyphenols in red wines has been estimated to range from 2000 to 6000 mg/L (Quideau, Deffieux, Douat-Casassus, & Pouysegu, 2011). Wine polyphenols have been reported to be bioavailable in several studies (Bitsch, Netzel, Frank, Strass, & Bitsch, 2004; Gonthier et al., 2003; Nardini et al.,

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2009; Vitaglione et al., 2005). These compounds are directly related to the quality of wines, so phenolic analysis can be used as an effective tool in characterizing different wines. Many factors can influence the phenolic composition of wines, including grape variety and the technology applied (Mulero, Zafrilla, Cayuela, Martinez-Cacha, & Pardo, 2011). Nevertheless, wine may also contain some components negatively affecting the health of moderate wine drinkers, such as pesticides, preservatives (sulfur dioxide, sulfites), trace metals and various compounds produced by different microorganisms during winemaking, including neurotoxins (ochratoxin A), potential carcinogens (ethyl carbamate) and allergens (biogenic amines) (Pozo-Bayon, Monagas, Bartolomé, & Moreno-Arribas, 2012). Among these, sulfur dioxide and sulfites are widely used during the different steps of winemaking and storage, for their sterilizing and antibacterial properties. Moreover, for their antioxidant properties, they can act against non-enzymatic and enzymatic oxidation of wines. Although sulfur dioxide and sulfites are widely used as preservatives in food, beverage and pharmaceutical industries, the use of these additives is strictly controlled due to the risks for human health derived from their consumption. In addition, high doses of sulfur dioxide/sulfites can cause organoleptic alterations in the final product (indesirable aromas of the sulfurous gas and the reduction products hydrosulfate and mercaptans). Sulfites may induce relevant adverse effects after their ingestion, such as anaphylactic shock, asthmatic attacks, urticaria, angioedema, nausea, abdominal pain, diarrhea and even death (Dalton-Bunnow, 1985; Vally & Thompson, 2003; Yang & Purchase, 1985). A considerable percentage of consumers show intolerance or high sensitivity to sulfites, with increasing risk in asthmatic and children. Recently, Laggner, Hermann, Sturm, Gmeiner, and Kapiotis (2005) reported that sulfite at concentrations found *in vivo* strongly promote low-density lipoprotein (LDL) oxidation by $Cu^{2+}$ and stimulate the LDL-oxidase activity of ceruloplasmin, acting as a pro-atherogenic agent in the presence of transition metal.

The consumption levels of sulfites show that the risk of exceeding the Acceptable Daily Intake concerns only regular consumers of alcoholic beverages, such as cider, beer and, particularly, wine, the main vector (Bemrah et al., 2012; Mareschi, Francois-Collange, & Suschetet, 1992).

According to European Commission regulations (Ruling n° 606/2009) (EC, 2009), the total sulfur dioxide content cannot exceed 150 mg/L in conventional red wines, and 200 mg/L in conventional white wines. In organic wines, the total sulfur dioxide content cannot exceed 100 mg/L in red wines and 150 mg/L in white wines. These legislative rules, attracted the interest of scientific research to reduce and replace the amount of sulfites in wines. Methods for sulfites removal or reduction during wine-making, as absorption through the use of anion and cation exchangers or membranes, the use of electrochemical treatments, lysozyme, bacteriocins or other chemical additives such as dimethyldicarbonate have been described (Garcia-Ruiz et al., 2008; Pozo-Bayon et al., 2012). Recently, special attention has been paid to the potential use of some natural constituents of grapes and wines, such as phenolic compounds, as an alternative to sulfites (Garcia-Ruiz, Moreno-Arribas, Martin-Alvarez, & Bartolomé, 2011). Nevertheless, more studies are necessary to evaluate the potential use of these technologies in winemaking.

In the recent years, wines produced using an environmentally sustainable approach, such as organic wines, have enjoyed increasing popularity, due to the growing demands for healthy products. In particular, the production of organic wines with no added sulfur dioxide/sulfites has gained considerable interest.

This study aims to investigate the total polyphenols and flavonoids (a class of polyphenols family) content, the phenolics profile and the antioxidant activity of organic red wines obtained without sulfur dioxide/sulfites addition in comparison to those of conventional red wines.

## 2. Materials and methods

### 2.1. Materials

Caffeic acid, catechin, syringic acid, *p*-coumaric acid, ferulic acid, resveratrol, myricetin, quercetin, trolox, gallic acid, potassium peroxodisulfate, 2,4,6-tris(2-pyridyl)-S-triazine, 2,2′-azino-bis(3-ethyl benzothiazoline-6-sulfonic acid) diammonium salt (ABTS) and EDTA were from Sigma (St. Louis, MO, USA). Rutin, hydroxytyrosol and isoferulic acid were from Extrasynthese (Genay Cedex, France). Ascorbic acid and all organic solvents were obtained from Carlo Erba (Milano, Italy). Stock solutions of standard phenolics were prepared in methanol (1 mg/ml), stored at −80 °C and used within 1 week. Working standard solutions were prepared daily by dilution in sample buffer (1.25% glacial acetic acid, 7% methanol in twice-distilled water).

### 2.2. Wines

Both organic and conventional red wines used in this study were produced in Italy and purchased at local markets and wine shops. All organic wines were produced according to the official organic farming practices (Italian Association for Organic Farming, AIAB, Italy), which typically excludes the use of artificial chemical fertilizers, pesticides, fungicides and herbicides, and have certificate of organic production. Although vinifications were performed before the final approval of the Reg. EC 203/2012, the winemaking protocol used suited the requisites of the EU regulation for organic wine production (EC, 2012). All the organic red wines analyzed in this study were obtained without sulfur dioxide/sulfites addition during winemaking processes.

Wine bottles were stored in the dark and analyzed immediately after opening, upon appropriate dilution (in the range 125–2000 fold dilution in final samples). Aliquots were frozen at −80 °C for phenolics determination.

### 2.3. Wines analyses

Total acidity and total sulfur dioxide were measured according to European official methods (EC, 1990).

Total polyphenols were evaluated by the Folin–Ciocalteu method (Singleton & Rossi, 1965), using gallic acid as reference compound. Results are expressed by reference to the calibration curve as milligrams of Gallic Acid Equivalents per liter of wine.

The total flavonoids content was determined by a colorimetric method previously described (Dewanto, Wu, Adom, & Liu, 2002), using catechin as reference compound. Results are expressed by reference to the calibration curve as milligrams of catechin equivalents per liter of wine.

### 2.4. Wine treatment for phenolics determination by high performance liquid chromatography (HPLC)

Wine (1 ml aliquots) was added with isoferulic acid (40 μg) as internal standard and NaCl (300 mg) and phenolic compounds extracted with diethylether and diethylacetate as described by Pozo-Bayon, Hernandez, Martin-Alvarez, and Polo (2003). Then, the extract was evaporated under nitrogen stream. For total phenolic acids determination (caffeic acid, ferulic acid, *p*-coumaric acid and syringic acid), alkaline hydrolysis treatment in the presence of ascorbate and EDTA was performed on wine sample prior to the extraction procedure (Nardini, Cirillo, Natella, & Scaccini, 2002).