



## Replacement of sulfur dioxide by hydroxytyrosol in white wine: Influence on both quality parameters and sensory



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### ABSTRACT

The feasibility of two hydroxytyrosol commercial products as an alternative to sulfur dioxide (SO<sub>2</sub>) in Sauvignon wines was evaluated. The hydroxytyrosol enriched products came from synthesis and olive waste. For this purpose wines elaborated with those products were compared with control ones elaborated with SO<sub>2</sub>. Enological parameters, color related parameters, antioxidant activity, volatile compounds, sensory analysis and olfactometric profile were determined in wines. Moreover, the evolution of wines after bottling was evaluated over six months. No significant differences were found in enological parameters and volatile composition (esters, alcohols and acids). However, significant differences were observed in color related parameters, antioxidant capacity, sensory analysis and olfactometric profile. Hydroxytyrosol-enriched wines resulted more colored and with higher antioxidant activity. Their main sensorial attributes did not correspond with the typical for Sauvignon blanc wines, which was related with a decrease in the odor intensity of the volatile thiol 3-mercaptohexyl acetate.

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### 1. Introduction

The most widely preservative used in wine industry is sulfur dioxide (SO<sub>2</sub>). Its antioxidant and antimicrobial properties make it essential nowadays. SO<sub>2</sub> has been used to inhibit polyphenol oxidase activity during winemaking, as well as to control the onset of undesirable fermentations such as acetic or malolactic fermentation (Guerrero & Cantos-Villar, 2015). However, the use of SO<sub>2</sub> has also drawbacks. Several human health risks, including dermatitis, urticarial, angioedema, diarrhea, abdominal pain, bronchoconstriction and anaphylaxis, have been associated to SO<sub>2</sub>. It is important to reduce the amount of SO<sub>2</sub> in wine since this compound is found in many food products as a food preservative and the amount consumed is accumulated in the organism (Vally, Misso, & Madan, 2009). Consequently, the International Organization of Vine and Wine (OIV) has been progressively reducing the

maximum SO<sub>2</sub> recommended concentration authorized in wines, which is nowadays 210 mg/L for white and rosé wines and 160 mg/L for red wines (OIV, 2012). Moreover, SO<sub>2</sub> addition in wine can produce organoleptic alterations in the final product, neutralize the aroma and even produce characteristic aroma defects.

For above reasons the research on alternatives to SO<sub>2</sub> has been enhanced. Some emerging technologies, also called green technologies, have been proposed as possible alternatives to SO<sub>2</sub>. Pulsed electric field, ultrasounds, high pressure and ultraviolet light have been tested in wines. However, further research is necessary to know in more detail the effect of those treatments on the sensorial properties of wines, and to validate the applicability of these technologies in wineries. Some chemical compounds have been also investigated: colloidal silver complex, dimethyl dicarbonate, ascorbic acid, hypophosphorous acid, thioldipropionic acid, Trolox C, stannous chloride, Sporix, sodium hypochlorite and even natural products such as lysozyme and bacteriocins (Santos, Nunes, Saraiva, & Coimbra, 2012). Among them, the use of phenolic compounds has been proposed as a promising alternative. For example, enological

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tannins combined with lysozyme were added in alcoholic fermentation (Sonni, Cejudo Bastante, Chinnici, Natali, & Riponi, 2009), and rich extracts in polyphenols from almond skin and eucalyptus leave have been proved in Verdejo wines during aging in barrels (González-Rompinelli et al., 2013). However an alternative which completely substitute SO<sub>2</sub> in winemaking has not been found yet.

Hydroxytyrosol (HT) is a phenylethyl alcohol which shows high antioxidant and antimicrobial capacity. HT is naturally found in wine in a wide concentration range. In white wines, values ranged from 1.75 to 45 mg/L (Fernández-Mar, Mateos, García-Parrilla, Puertas, & Cantos-Villar, 2012). HT, among other olive oil polyphenols, has been recently accepted as protective compound against oxidative damage (EFSA, 2011). In a previous study the antioxidant activity, antimicrobial activity and olfactometric profile of an olive mill waste extract with high HT concentration was evaluated (Ruiz-Moreno et al., 2015). It was concluded that the extract was a suitable source of both antioxidants and antimicrobials, although its odorants may contribute negatively to wine. Based on these results, in the current work two different HT extracts, were tested as a possible alternative to SO<sub>2</sub> in white wine.

The aim of this study was to evaluate the feasibility of hydroxytyrosol as an alternative to SO<sub>2</sub> in white wines. Enological quality parameters, color related parameters, antioxidant capacity, volatile composition, olfactometric profile and sensory wine properties were evaluated.

## 2. Materials and methods

### 2.1. Chemicals

Analytical grade methanol and formic acid were supplied by Panreac (Barcelona, Spain). Chemical standards: hydroxytyrosol, Trolox (6-hidroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), 2,20-azobis(2-amidinopropane) dihydrochloride (AAPH), 2,2-diphenyl-1-picrylhydrazyl (DPPH), K(OH) solution, dichloromethane (LiChrosolv quality), aroma standards and alkane solution (C7–C40) used for identification were purchased from Sigma–Aldrich (Steinheim, Germany). Anhydrous sodium sulfate was obtained from Panreac (Barcelona, Spain). Ultrapure water from a Milli-Q system (Millipore Corp., Bedford, MA) was used throughout this research.

### 2.2. Hydroxytyrosol commercial products

Two products based on HT were used in the present study. The first, HT product was produced by chemical and enzymatic synthesis with analytical purity (>99%) (SeproX Biotech, Spain), hereinafter called as HTB. It is generally recognized as safe (GRAS). The second one, was a natural extract from olive byproducts, whit a richness of 26% HT (Hytolive<sup>®</sup>, Genosa I + D, Spain), hereinafter referred as HTG.

### 2.3. Winemaking

A 600 kg of Sauvignon blanc grapes were harvested, destemmed, crushed and pressed. After pressing, enzymes were added into the must (2.5 mL/hL, Enartis ZYM Blanco L, Italy), dejuiced (24 h at 4 °C) and placed in a 300 L steel vessel, which had been previously filled up with nitrogen. Alcoholic fermentation (AF) was then started after yeasting (Aroma White, Italy). AF was developed during 13 days under control temperature (18 °C). CO<sub>2</sub> was added daily to assure reductive conditions. Afterward, wine was kept cold (15 °C) under nitrogen atmosphere for another 13 days until sugar concentration was under 3 g/L. Then, the wine was divided in three

batches, each one in triplicate. 80 mg/L of SO<sub>2</sub> (Solfosol, Sepsa-Enartis, Spain) were added to CT wines, 80 mg/L of HT synthetic to HTB wines, and 308 mg/L of Hytolive (for 80 mg/L of HT) to HTG wines. This concentration was selected in agreement with antioxidant activity of hydroxytyrosol (Ruiz-Moreno et al., 2015) and previous sensory studies (data not shown).

Wines were stabilized in a cold chamber during two months. Subsequently, the wines of each batch were racked, filtered (Optical XL 10W, Millipore, France) and bottled. Bottled wines were stored under control conditions (16 °C, 80% RH) during 6 months. Sampling was conducted after addition of antioxidants (end of AF), at bottling and after six months of storage in bottle.

### 2.4. Enological parameters

Density, ethanol, glycerine, dry extract, total and volatile acidity, pH, SO<sub>2</sub>, organic acids (acetic, citric, tartaric, malic, lactic and succinic acids) and metals (Na, K, Ca, Cu, Fe and Zn) were determined at bottling following the official analytical methods established by the International Organization of Vine and Wine (OIV, 2012).

### 2.5. Color related parameters

Color intensity (D.O. 420 nm + D.O. 520 nm) and hue (D.O. 420 nm/D.O. 520 nm) were determined by spectrophotometric measures (Lambda 25, Perkin–Elmer, USA). Colorimetric measurements were registered with a Konica-Minolta CM-3600d spectrophotometer (Osaka, Japan), using 20 mm path length glass cells and distilled water as reference. The CIELab parameters ( $L^*$ ,  $a^*$ ,  $b^*$ ) were determined by using the software SpectraMagic v.3.61G (Cyberchrome Inc, Minolta Co. Ltd), following the recommendations of the Commission Internationale de L'Eclairage (CIE): the standard observer (D10°) and the standard illuminant (D65). Color differences ( $\Delta E^*_{ab}$ ) were calculated as the Euclidean distance between two points in the 3D space defined by  $L^*$ ,  $a^*$ , and  $b^*$  (Martínez, Melgosa, Pérez, Hita, & Negueruela, 2001).

### 2.6. HPLC determination of hydroxytyrosol

Hydroxytyrosol was quantified as described by authors (Piñeiro, Cantos-Villar, Palma, & Puertas, 2011). Briefly, 20 µL of must or wine were analyzed by a Jasco high-performance liquid chromatographic system equipped with a diode array detector (model MD-2010), a fluorescence detector (model FP-2020), an HPLC pump module (model PU-2089), a column oven module (model CO-2060) and an auto-sampler module (AS-2050), controlled by Chrompass version 1.8 software.

### 2.7. Analysis of volatile compounds by gas chromatography

The analysis of wines fermentative volatile compounds was performed by the method described by Ortega, López, Cacho, and Ferreira (2001) with modifications (Garde-Cerdán et al., 2014) after 3 months of bottling. The extracts were injected onto a Hewlett–Packard (Palo Alto, CA) 6890 gas chromatograph equipped with an automatic injector and a Hewlett–Packard FID detector. Separation was carried out with a DB-Wax capillary column (60 m × 0.32 mm I.D. × 0.5 µm film thickness; J&W Scientific, Folsom, CA). The temperature program was: 40 °C for 5 min then raised up to 220 °C at a rate of 3 °C/min. The carrier gas was nitrogen at a flow rate of 3 mL/min. Injector temperature was 220 °C and detector temperature was 280 °C. Identification of compounds was carried out by comparison of their retention times with those of pure reference standards.

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