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Effect of hydroxytyrosol on quality of sulfur dioxide-free red wine

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ABSTRACT

In this work, the feasibility of two commercial products enriched in hydroxytyrosol (HT) as alternative to sulfur dioxide in Syrah red wines was evaluated. The HT enriched products came from synthesis and from olive waste. Wines treated with HT were compared with wines treated with sulfur dioxide at two winemaking stages: bottling and after 6 months of storage in bottle. Minor differences were found in enological parameters and volatile composition (esters, alcohols and acids). Significant differences were observed in color related parameters and sensory analysis. HT wines improved color parameters as well as scents and tasting at bottling. However, after 6 months of storage in bottle HT wines were more oxidized than SO_2 wines. The olfactometry profile of HT wines supported sensory analysis. HT wines showed new odorant zones from both the added product and oxidation.

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1. Introduction

The most widely preservative used in the wine industry is sulfur dioxide (SO_2). Its antioxidant and antimicrobial properties make it essential nowadays. SO_2 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_2 has also drawbacks. Several human health risks, including dermatitis, urticarial, angioedema, diarrhea, abdominal pain, bronchoconstriction and anaphylaxis, have been associated with SO_2. Furthermore, it is also important to reduce the amount of SO_2 in wine since this compound is found in many food products as a food preservative and the amount consumed is accumulative in the organism (Vally, Misso, & Madan, 2009). In sulfite-sensitive individuals, allergic reactions could be severe, since SO_2 derivatives can cause the activation of proto-oncogenes, inactivation of tumor suppressor genes, and even can play a role in the pathogenesis of SO_2 associated lung cancer. Thus, increasingly, consumers have been clamoring for natural, organic alternatives as opposed to the chemical preservatives present in wine. In fact, there are negative perceptions of sulfites and willingness to pay for non-sulfited wines (Costanigro, Appleby, & Menke, 2014).

From an enological point of view, SO_2 can produce organoleptic alterations in the final product, neutralize the aroma and even produce characteristic aroma defects (Ribereau-Gayon, Dubourdieu, Doneche, & Lonvaud, 2006). Additionally, only molecular SO_2 (a fraction of the free sulfite present) possesses antioxidant and antimicrobial properties and its percentage depends on the wine pH. High pH decreases its proportion, and therefore its effectiveness.

Some emerging technologies, also called green technologies, have been proposed as possible alternatives to SO_2. Pulsed electric field, ultrasounds, high pressure and ultraviolet light have been tested in wines. However, it is still necessary 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, thiodipropionic acid, Trolox C, stannous chloride, and Sporix, sodium hypochlorite and even natural products such as lysozyme and bacteriocins (Santos, Nunes, Saraiva, & Coimbra, 2012). However, there is not currently any substance or treatment that substitutes entirely the use of SO_2.

Hydroxytyrosol (HT) is a phenyl ethyl alcohol which shows high antioxidant and antimicrobial capacity. HT is naturally found in red

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wine between 1.98 and 3.89 mg/L (Fernández-Mar, Mateos, García-Parrilla, Puertas, & Cantos-Villar, 2012). HT 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 in wine model (Ruiz-Moreno et al., 2015). It was concluded that the extract was a suitable source of both antioxidants and antimicrobials, although its odorants might contribute negatively to wine. In the present work, two HT-enriched products were tested as a possible alternative to SO_2 in red wine. The aim of this study was to evaluate the feasibility of hydroxytyrosol as an alternative to SO_2 in red wines. Enological quality parameters, color related parameters, 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, 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 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, with a richness of 26% hydroxytyrosol (Hytolive®, Genosa I + D, Spain), hereinafter referred as HTG.

2.3. Winemaking

A complete diagram of the process is shown in Fig. 1. Syrah grapes (560 kg) were harvested, destemmed, crushed and placed into a Ganimede fermenter (Ganimede®). The design of this type of fermenter permits CO_2 to accumulate. Once the room is saturated with CO_2 gas, the excess of gas under pressure rises in big bubbles to the surface. This methodology is very effective in the extraction of phenolic compounds during winemaking as the CO_2 bubbles constantly agitate the mass of marc and keep the skins wet and evenly dispersed. Additionally, Ganimede system was selected since a reductive environment is generated inside the tank, which preserves the must from oxidation.

Alcoholic fermentation (AF) was started after yeast addition (20 g/hL, ES488, Sepsa-Enartis, Spain), which proceeded for 8 days at a controlled temperature (23 ± 1 °C). Malolactic fermentation was induced with *Oenococcus oeni* (1 g/hL, Challenge Easy ML, Sepsa-Enartis, Spain) and nutrients (20 g/hL Nutriferm ML, Sepsa-Enartis, Spain). Afterwards, the solid parts were placed in a pneumatic press (Willmes, Germany) and pressed. Free run wine and pressed wine were mixed. Then, wine was divided in three batches, each one in triplicate. 50 mg/L of SO_2 (Solfosol, Sepsa-Enartis, Spain) were added to CT wines, 50 mg/L of hydroxytyrosol synthetic was added to HTB wines, and 192 mg/L of Hytolive (for 50 mg/L of HT) was added to HTG wines. Subsequently, wines were stabilized in a cold chamber (at 0 °C) during 2 months. Finally, the wines of each batch were racked, filtered (Optical XL, Millipore, France) and bottled. Bottled wines were stored under controlled conditions (16 °C and 80% HR) for 6 months. Wine sampling was conducted after addition of antioxidants (end of AF), at bottling and after 6 months of storage in bottle.

2.4. Enological parameters

Relative density, ethanol, glycerin, dry extract, total and volatile acidity, pH, total and free SO_2, organic acids (acetic, citric, tartaric, malic, lactic, and succinic acids), metals (Na, Ca, K, Fe, Cu and Zn), anthocyanin, tannin and total polyphenols index (TPI) 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 + D.O. 620 nm) and hue (D.O. 420 nm/D.O 520 nm) were determined by spectrophotometric measures (Lambda 25, Perkin-Elmer, Massachusetts). Colorimetric measurements were registered with a Konica-Minolta CM-3600d spectrophotometer (Osaka, Japan), using 2-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'Eclariage (CIE): the standard observer (D10°) and the standard illuminant (D65). Color differences (ΔE^*_{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 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. The column used was a Mediterranea Sea-C18 column (RP-18, 250 × 4.6 cm; 5 μm particle size) from Teknokroma (Barcelona, Spain) with a guard column made of the same material. The mobile phase consisted of A (water–formic acid 99.9–0.1%) and B (methanol–formic acid 99.9–0.1%).

2.7. Analysis of volatile compounds by gas chromatography

The analysis of wine fermentative volatile compounds was performed using the method described by Ortega, López, Cacho, and Ferreira (2001) with modifications (Garde-Cerdán et al., 2014) after 4 months of bottling. The extraction was carried out by mixing 3 mL of sample, 9.5 mL of $(NH_4)_2SO_4$ saturated solution, 15 μL of internal standard solution (2-butanol, 4-methyl-2-pentanol, 4-hydroxy-4-methyl-2-pentanone, 2-octanol, and heptanoic acid, 40 mg of each of them/100 mL of ethanol) and 200 μL of dichloromethane in tubes. The tubes were shaken for 1 h at 400 rpm and then centrifuged at 2500 rpm for 10 min. Once the phases were separated, the dichloromethane phase was recovered. 2 μL of this extract were injected onto a Hewlett-Packard (Palo Alto, CA) 6890 series II 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, USA). The

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