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A winery-scale trial of the use of antimicrobial plant phenolic extracts as preservatives during wine ageing in barrels

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ABSTRACT

Antimicrobial plant extracts rich in polyphenols have recently been proposed as a total or partial alternative to sulfites during winemaking. This paper reports a first winery-scale trial of the addition of antimicrobial plant extracts during wine ageing in wood. Before being distributed in oak barrels, a Verdejo wine was treated with either a SO_2 regular dose (160 mg/L) or with a SO_2 half-dose (80 mg/L), together with two phenolic-rich extracts from eucalyptus leaves and almond skins (100 mg/L). Some of the wine was also stored in a stainless steel tank for comparison. After 6 months of ageing, the wine treated with the phenolic extracts remained microbiologically stable and showed correct enological parameters. Also, the volatile and phenolic composition of the wine was specifically determined to ascertain whether the addition of these phenolic extracts would affect the organoleptic properties of the wine. Although the addition of both eucalyptus and almond extracts led to statistically significant changes ($p < 0.05$) in the concentration of several esters, C13 norisoprenoids, volatile phenols and furanic compounds in the wine, only the concentration of some of these compounds was higher than their odor threshold. With regard to phenolics, addition of both extracts did not significantly modify their content, except for a lower content of hydroxycinnamic acids and esters and flavan-3-ols, which predicts minor changes in wine astringency. As seen from a PCA analysis of all volatile and phenolic data, wines were mainly differentiated by the ageing process itself, by the addition of extracts, and even by the barrel used. Finally, a triangle test showed no significant differences in the global sensory appreciation between wines treated and not treated with the antimicrobial phenolic extracts. These results demonstrated the potential applicability of phenolic extracts as a partial alternative to sulfur dioxide during the ageing of white wines in oak barrels.

1. Introduction

Sulfurous anhydride or sulfur dioxide (SO_2) is a widely employed preservative used to protect wine from alterations. SO_2 has multiple and diverse properties such as antioxidants and selective antimicrobial effects, especially against lactic acid bacteria (LAB). However, its use in winemaking is strictly controlled, since high doses can cause organoleptic alterations in the final product, and, in particular, because of human health risks such as headache, allergic reaction, fatigue, asthma, itching, etc. (Taylor, Higley, & Bush, 1986). Thus, there is a great interest in looking for other preservatives and innovative technologies, harmless to health, that can replace or at least complement the action of SO_2, making it possible to reduce its levels in wines (Santos, Nunes, Saraiva, & Coimbra, 2012). As alternatives, physical treatments (pulsed electronic fields, UV irradiation and ultrasound, among others) have been considered (Fredericks, du Toit, & Krügel, 2011; Lustrato et al., 2010), as well as the addition of compounds such as bacteriocins, dimethyl dicarbonate (DMDC) and lysozyme (Santos et al., 2012). Most recently, the effectiveness of a colloidal silver complex as an antiseptic instead of SO_2 in winemaking has also been reported (Izquierdo-Cañas, García-Romero, Huertas-Nebreda, & Gómez-Alonso, 2012).

Several studies have proven the inhibitory effects of pure phenolic compounds in the growth of isolated LAB (for a review see

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García-Ruiz et al., 2008) as well as the effective inhibitory concentrations and mode of action of these promising antimicrobial natural compounds (Campos, Couto, & Hogg, 2003; García-Ruiz, Bartolomé, Cueva, Martín-Álvarez, & Moreno-Arribas, 2009; García-Ruiz, Moreno-Arribas, Martín-Álvarez, & Bartolomé, 2011). Moreover, a recent study has shown that some plant phenolic extracts were able not only to inhibit the growth of enological LAB but also to delay malolactic fermentation in red wines on a laboratory scale (García-Ruiz et al., 2012). In the developing of new alternatives to the use of sulfites in enology, experiments on an industrial-scale that confirmed the results obtained in the laboratory are essential and the definitive step toward demonstrating the technological application and its impact on wine properties and quality.

SO_2 is certainly a very effective preservative added to wine after fermentation and before ageing to ensure microbial stability and to achieve better control of the ageing process. Premium-quality wines with a complex aromatic structure can gain in quality from maturation time. In many winemaking regions, 'wood ageing', the oxidative ageing of wines in wooden barrels, is a traditional practice. From a chemical point of view, the ageing process specifically modifies the volatile and phenolic composition of white wines. Among volatile compounds, lactones, furanic and vanillin compounds, which are mainly generated during the wood-toasting process (Díaz-Plaza, Reyero, Pardo, Alonso, & Salinas, 2002), form the most important groups of aromas in wines obtained by ageing in wood. Wine ageing in barrels is characterized by a slow and continuous penetration of oxygen through the wood causing the oxidation of the wine constituents, particularly polyphenols, and subsequent changes in astringency, color and taste (Singleton, Orthofer, & Lamuela-Raventós, 1998).

The hypothesis behind this study was that the addition of antimicrobial phenolic extracts could also be effective in the preservation of industrially manufactured white wines during their ageing in barrels, which would reduce, at least partly, the amount of SO_2 added to wines. Bearing this in mind, we have evaluated the addition of two phenolic extracts, eucalyptus leaves and almond skins, whose antimicrobial properties have been previously proved (García-Ruiz et al., 2012), as a partial alternative to SO_2 during the ageing of Verdejo wine in oak barrels. *Vitis vinifera* cv. Verdejo is an important Spanish white cultivar characteristic of the Denomination of Origin Rueda located in the region of Castilla y León (Spain) (Martínez-Gil, Garde-Cerdán, Martínez, Alonso, & Salinas, 2011). This variety is mainly used to produce young white wines with a fruity aroma and flavor, although, in recent years, in an attempt to obtain wines of a more complex quality, Verdejo wine has been subjected to ageing in oak barrels (Rodríguez-Nogales, Fernández-Fernández, & Vila-Crespo, 2009). In order to evaluate their microbial stability, wines were analyzed for colony counting and main enological parameters, after ageing in barrels. The volatile and phenolic composition of wines was specifically determined to ascertain whether the addition of these phenolic extracts would affect the wines' organoleptic properties. Finally, triangle tests were carried out by peer tasters to see whether there were any significant differences in the global sensory appreciation of the wines treated and those not treated with the antimicrobial phenolic extracts.

2. Materials and methods

2.1. Reagents and solvents

Pure volatile compounds were supplied by Aldrich (Gillingham, UK), Fluka (Buchs, Switzerland), Riedel-de Haën (Seelze, Germany) and Firmenich (Geneva, Switzerland). Pure phenolic compounds were purchased from Sigma (St. Louis, MO, USA), Extrasynthèse (Genay, France), Phytolab (Vestenbergsgreuth, Germany) and Scharlau (Barcelona, Spain). Commercial phenolic extracts from eucalyptus leaves and almond skins were kindly provided by their producer, Biosearch Life S. A. (Granada, Spain). The eucalyptus extract contains gallic acid and flavonols (García-Ruiz et al., 2013) whereas the almond extract is rich in flavan-3-ols (Garrido, Monagas, Gómez-Cordovés, & Bartolomé, 2008).

2.2. Winemaking process and treatments

A white wine (var. *Verdejo*) (vintage 2010) was elaborated at Bodegas José Pariente S.L. (Valladolid, Spain), following their own winemaking procedures. The alcoholic fermentation was carried out in a controlled form in stainless steel tanks (10,000 L) by commercial active dry yeast at 13 ± 2 °C. The end of alcoholic fermentation was established by measuring the alcohol degree (13.9% v/v) and the residual sugar amount (<4 g/L); the wine pH at the end of alcoholic fermentation was 3.25. Once alcoholic fermentation was completed, the wine was distributed into different 225 L oak barrels, in which the different treatments were carried out. Treatments were as follows: 160 mg/L SO_2 (usual dose SO_2 in white wine) (control wine), 80 mg/L SO_2 + 100 mg/L eucalyptus leaves extract (wine treated with the eucalyptus extract) and 80 mg/L SO_2 + 100 mg/L almond skins extract (wine treated with the almond extract). The concentration added of both phenolic extracts, 100 mg/L, was determined after analyzing their solubility and stability in a synthetic wine. Two barrels (#1 and #2) from the same manufacture lots and characteristics were used for each experiment with the antimicrobial extracts, whereas only one barrel was used for the control wine. In addition, part of the wine was stored in the initially used stainless steel tank after being treated with 160 mg/L SO_2. Wine samples were collected before ageing and after 2 and 6 months of ageing in tank/barrels at 13 ± 1 °C. Samples were analyzed in duplicate.

2.3. Enological parameters

Total and volatile acidity, pH (direct measurement by using a pH meter) and alcohol content were evaluated according to official or usual methods recommended by the International Organisation of the Vine and Wine (OIV, 1990). Wine samples were collected before and after ageing (6 months) in a tank/barrels. The analyses were performed in duplicate.

2.4. Microbiological analysis

Wine samples collected after ageing (6 months) were assayed for LAB colony counting. Samples were plated onto MRS-Agar (Pronadisa, Madrid, Spain), supplemented with 5 g/L fructose (Panreac Química SAU, Barcelona, Spain), 1 g/L D-L malic acid (Panreac Química SAU, Barcelona, Spain) and 1 mL Tween 80 (Sigma, St. Louis, USA). The pH of the medium was adjusted to 4.8 with 37% HCl (Panreac Química SAU, Barcelona, Spain). For the spot test, aliquots of 100 μL of wine samples were transferred to 900 μL of sterile saline and then submitted to serial 10-fold dilutions in sterile saline and 10 μL of each dilution were plated on the surface of plates containing MRS-Agar. The plates were incubated at 28 °C for 7 days. Colony counts were expressed as colony forming units (CFU) per mL of wine. All dilutions were realized in duplicate.

2.5. Volatile composition analysis

For the analysis of volatile compounds, 8 mL of wine sample, 40 μL of an internal standards solution (3,4 dimethylphenol, 400 mg/L; 3-octanol, 10 mg/L; and methyl nonanoate, 2.5 mg/L) and