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Effect of the aging on lees and other alternative techniques on the low molecular weight phenols of *Tempranillo* red wine aged in oak barrels

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ABSTRACT

The effect of different alternative techniques to the traditional aging on lees on the low molecular weight phenolic compounds of red wines was study as well as their evolution during the aging in oak wood barrels for six months. The study was carried out with *Tempranillo* red grapes from two consecutive vintages. The techniques assayed were the traditional aging on lees with or without the addition of exogenous β-glucanase enzymes, the use of yeast derivative preparations also with or without the addition of exogenous β-glucanase enzymes, the micro-oxygenation applied together with the aging on lees, and the use of non-toasted oak wood chips.

Hydroxycinnamic acids were the compounds most affected by these treatments, mainly in the wines treated with chips and commercial yeast derivative products, which showed higher concentrations of the free acids, compounds that play an important role in wine stabilization color since they can act as anthocyanin copigments.

The differences found between the assayed treatments were more important in the 2007 vintage than in the 2008. However, a more significant effect of micro-oxygenation in the 2008 vintage was observed, which could be related to the fact that in this vintage the treatment was longer. In the 2008 vintage, the differences between treatments decreased along the aging in barrel. This vintage effect could be associated to the differences in the phenolic concentration of the initial wines. In this sense more research should be done to corroborate this fact.

1. Introduction

During aging on lees, some interesting metabolites as such as mannoproteins and glucans can be released into wines due to yeast autolysis. The compounds released can interact with wine phenolic compounds, decreasing their astringency [1] and/or acting as protective colloids, enhancing the color stability of red wines [1–3]. Furthermore, Riou et al. [4] and Poncet-Legrand et al. [5] observed in model wine solutions that some high molecular weight polysaccharides prevented tannin aggregation. The hypothesis proposed by these authors to explain this fact is that this type of polysaccharides can bind proanthocyanidins to give rise to more stable aggregates that prevent their polymerization and precipitation, acting as protective colloids. The presence of these polysaccharide-tannin complexes can reduce astringency and increase roundness, structure and mouth-feel of wines [4–7]. Mannoproteins can also contribute to wine color stabilization due to their capacity to interact with tannins and anthocyanins preventing their aggregation and precipitation [2].

However, one of the disadvantages of aging on lees is that consumes oxygen. Oxygen plays an important role in the condensation reactions between flavonoids mediated by acetaldehyde and/or glioxylic acid [8,9], and in the cicloaddition reactions between pyruvic acid and anthocyanins [10]. As result of these polymerization and condensation reactions, new polymeric structures are formed that enhance wine sensorial characteristics such as color stability and astringency. Therefore, the consumption of oxygen can reduce the condensation and polymerization reactions between phenolic compounds, and also favors the formation of reduction aromas. The application of the micro-oxygenation technique together with the traditional aging on lees could avoid these problems since this technique consists in the addition of small and controlled amounts of oxygen [11]. In this way, micro-oxygenation combined with aging on lees could reduce the presence of reductive aromas [12,13] and could favor the formation or more stable colored pigments improving the color stability of red wines over the time [14,15]. The positive effects of this technique on wine color and the formation of new polymeric pigments have been corroborated by several authors [10,14–19].

On the other hand, aging on lees can be improved by the addition of exogenous β-glucanase enzymes to wines, accelerating the

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yeast autolysis process, and favoring the release of glucans and mannoproteins [20].

Nowadays, the use of commercial yeast derivative preparations from *Saccharomyces cerevisiae* has increased for the last three years. The goal of these preparations is to release the mannoproteins and glucans from the yeast cell walls more quickly into wines, reducing in this way the time needed to obtain wines with physico-chemical and sensory characteristics similar to those aged on lees. Some authors have studied the effect of these products on the volatile compounds [21–23], the color and anthocyanin pigments of wines [24–26]. However, few published papers have focused on studied the effect of commercial yeast preparations on the non anthocyanin phenolic compounds of red wines. Only Guadalupe and Ayestarán [24] and Guadalupe et al. [25] studied the effect of commercial mannoproteins on some non anthocyanin phenolic compounds of red wines such as hydroxycinnamic acids and flavanols.

Other vinification technique that gives rise to red wines with sensory characteristics similar to the wines aged on lees is the use of non-toasted wood chips after alcoholic fermentation and before the beginning of the malolactic fermentation. This fact is due to the polysaccharides [27,28], and the phenolic compounds that are released into wines from wood, and which can also interact with wine phenolic compounds.

Non anthocyanin phenolic compounds of wines play also an important role in color and taste characteristics of wines. They can be classified as phenolic acids (hydroxybenzoic and hydroxycinnamic acids and their derivatives), flavanols, flavonols, stilbenes and phenolic alcohols. The hydroxycinnamic acids, flavanols and flavonols can act as copigments in copigmentation reactions with anthocyanins, improving in this way the color of mainly young red wines [29–31].

Besides, in the last years, the non-anthocyanin phenolic compounds, especially flavonols and stilbenes, have been recognized by their importance in the health human due to their high antioxidant activity, anticarcinogenic potential, anti-inflammatory properties and because they can prevent cardiovascular diseases [32–36].

For all these reasons, the aim of this work was to study the effect of different alternative techniques to the traditional aging on lees on the low molecular weight (non anthocyanin) phenolic compounds of red wines and to study their evolution during the aging in oak wood barrels for six months. The study was carried out in two consecutive vintages. The techniques assayed were the traditional aging on lees with or without the addition of exogenous β-glucanase enzymes, the use of yeast derivative preparations also with or without the addition of exogenous β-glucanase enzymes, the micro-oxygenation applied together with the aging on lees, and the use of non-toasted oak wood chips.

2. Material and methods

2.1. Winemaking process and treatments

The study was carried out using *Tempranillo* red grapes from Cigales Designation of Origin sited in the Autonomous Community of Castilla y León in the North of Spain, from two consecutive vintages (2007 and 2008). The red wines were elaborated in the research winery of the Enological Station of Castilla y León, following the traditional red winemaking process.

The grapes (about 12,500 kg) were harvested manually on the optimum harvest date, based mainly on the relation sugar content (°Brix) and total acidity, and transported to the Enological Station in 15-kg-plastic boxes. The clusters were de-stemmed and crushed with minimum physical damage. The mass obtained, was slightly sulphited ($0.04\,g\,L^{-1}$) and then transferred to five 2600 L stainless steel tanks to undergo alcoholic fermentation at controlled temperature (25–28 °C). Alcoholic fermentation was carried out through the inoculation with commercial yeast *S. cerevisiae* (Excellence sp, Lamothe-Abiet). Once the alcoholic fermentation was completed, the mass was pressed and the wines were kept in the tanks for 4 days to allow for the sedimentation of the gross lees. After this time, the wines were racked off and maintained in the tanks for 4–5 days to allow for the sedimentation of the fine lees. The base wine was then again racked off, homogenized and distributed into several 500 L tanks, except for the micro-oxygenation treatments in which special tanks were used. These tanks are 3 m high, since at least this height is necessary to get a good dissolution of the oxygen applied with the micro-oxygenation. The wet fine lees decanted in the bottom of the tanks were colleted and used in the experiments with lees.

The experiments carried out in the 2007 and 2008 vintage are shown in Fig. 1. In the case of the 2007 vintage, the following experiments were carried out in duplicate: control wines (without the addition of any product) (C); wines aged on lees (L); wines aged on lees and with the addition of commercial β-glucanase enzymes (L+E); wines aged on lees with micro-oxygenation (L+MO); wines with a commercial yeast derivative added (YD); wines with the same commercial yeast derivative and the commercial β-glucanase enzymes added (YD+E); and wines with non-toasted French oak chips added (CH).

Considering the results obtained in the 2007 vintage, and those obtained in a previous study relating to the use of β-glucanase enzymes [23,37,38], and taking into account the high number and variety of commercial yeast preparations that are appearing in the market, different studies were carried out in the 2008 vintage. In this vintage, three commercial yeast derivative products with different composition, purity and effect on wines were studied (Fig. 1). The aging on lees combined with the micro-oxygenation and the use of non-toasted oak chips were also studied.

Table 1 shows the information provided by the commercial manufactures regarding the commercial products used in this study, and the doses applied, in accordance with the manufactures' recommendations.

Micro-oxygenation was carried out by means of a modular five-head VisiO2 micro-oxygenator (Oenodev, France). In the two vintages studied, the same doses were applied: $5\,mL\,L^{-1}\,month^{-1}$ of O_2. However, in the first vintage the length of the treatment was 35 days and in the second vintage was 60 days.

Two batonnages per week were performed on each wine. The temperature was maintained at 15 ± 1 °C. All treatments lasted 60 days, with the exception of the YD3 product that was added just before the barrel aging, because according to its manufacture it is a soluble product with direct action.

After the treatments, all the wines were racked off and inoculated with a commercial preparation of *Oenococcus oeni* (Viniflora, CHR Hansen, Denmark) to induce malolactic fermentation. After that, the wines were racked off into new American oak barrels with a medium–high degree of toasting (two barrels for each treatment and replicate, see Fig. 1).

The samples were analyzed immediately after the malolactic fermentation (end of treatment), and after three and six months of aging in barrels.

2.2. Chemical reagents

Gallic, syringic and *trans*-*p*-coumaric acids, *trans*-resveratrol and catechin were provided by Sigma–Aldrich (Steinheim, Germany); protocatechuic, vanillic, ellagic and *trans*-caffeic acids, tyrosol, tryptophol, myricetin and kaempferol by Fluka (Buchs, Switzerland); epicatechin, ethyl gallate, syringetin-3-glucoside, and quercetin by Extrasynthèse (Lyon, France).