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Yeast influence on the formation of stable pigments in red winemaking

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ABSTRACT

The anthocyanin profile of a wine greatly varies over time depending on many factors. In addition to color modifications due to changes in the chemical composition of wine, there may be some influence of the yeast strain used in fermentation. The main aim of this study is to identify and quantify the different ways in which yeast may influence on wine color and its stability, during red winemaking. Hydroxycinnamate decarboxylase activity was measured by the ability to transform the *p*-coumaric acid (HPLC–DAD). Acetaldehyde (GC–FID) and pyruvic acid (Y15 enzymatic autoanalyser) contents were monitored along fermentation. Stable pigments formation, including vitisins, vinylphenolic pyranoanthocyanins and flavanols-anthocyanins adducts, were analyzed by HPLC–DAD/ESI-MS. Moreover, the ability of adsorbing color molecules by yeasts' cell walls was assessed. It could be concluded that the strain used has substantial influence on the formation of stable pigments, and therefore, proper yeast selection is important to ensure the stability of the wine coloring matter.

1. Introduction

The color of wine is mainly due to the presence of anthocyanins in grape skins, called grape anthocyanins or monomeric anthocyanins. However, these forms of color are very unstable and quickly condense or combine to create more complex and more stable pigments (He et al., 2012a), also they can be degraded by oxidation. Thus, the anthocyanin profile of a wine may vary depending on many factors such as the grape variety and ripeness, winemaking techniques, yeasts used and aging time (Caridi, 2013).

Pyranoanthocyanins and flavanols-anthocyanins adducts are highly stable, resistant to sulfur dioxide bleaching and oxidative degradation, therefore they can significantly contribute to the color stability of red wines (Escribano-Bailón, Álvarez-García, Rivas-Gonzalo, Heredia, & Santos-Buelga, 2001; He et al., 2012b). However, most of the pyranoanthocyanins (except portisins, but in fact these compounds should be considered as vinyl-flavanol adducts of pyranoanthocyanins) are characterized by a more orange color compared to the red–purple hue of the genuine pigments (Rentzsch, Schwarz, & Winterhalter, 2007). Conversely, the initial small anthocyanin-acetaldehyde-flavonoid polymers are thought to enhance the violet shift so typical of young red wines (Boulton, 2001; Dallas, Ricardo-da-Silva, & Laureano, 1996a).

Acetaldehyde is the major aldehyde of the wine and can be synthesised by the yeasts or formed from ethanol by the chemical oxidation of the wine (Jackowetz, Dierschke, & Mira de Orduña, 2011). Its reaction with anthocyanins promotes their

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polymerization with flavanols (Dallas, Ricardo-da-Silva, & Laureano, 1996b; Francia-Aricha, Guerra, Rivas-Gonzalo, & Santos-Buelga, 1997). Moreover, by reacting with sulfur dioxide, acetaldehyde may liberate anthocyanins for polymerization with proanthocyanidins. In addition, acetaldehyde is a precursor of vitisin B (malvidin-3-*O*-glucoside-acetaldehyde) (Marquez, Serratosa, & Merida, 2013).

Pyruvic acid is a very important compound in wine formed by the metabolic activity of yeasts and lactic acid bacteria (Morata, Calderón, González, Gómez-Cordovés, & Suárez-Lepe, 2007), because, besides being the precursor of many compounds, is involved in the formation of stable pigments, such as vitisin A (malvidin-3-*O*-glucoside-pyruvate) (Asenstorfer, Markides, Iland, & Jones, 2003).

Vinylphenolic pyranoanthocyanins are condensation products between vinylphenols and anthocyanins (Morata, González, & Suárez-Lepe, 2007). Vinylphenols originate during fermentation by the action of hydroxycinnamate decarboxylase enzyme (HCDC) of yeasts, which is capable of transforming the hydroxycinnamic acids (*p*-coumaric, caffeic and ferulic).

Other secondary metabolites from yeast, such as acetone, acetoin, oxalacetic acid, acetoacetic acid and diacetyl, are also likely to react with free anthocyanins to form similar pyranoanthocyanins (He et al., 2012b).

Copigmentation is other phenomena affecting color in red wines. Molecular proximity between pigments anthocyanins and other molecules (some flavonoids, phenolic acids, metallic cations and others) working as copigments can increase color intensity hyperchromic effect and produce shift higher wavelengths (bathochromic effect). Molecules such as flavonols (myricetin, quercetin, kaempferol), hydroxycinnamic acids (coumaric, ferulic and caffeic) and flavanols (flavan-3-ol monomers and proanthocyanidins) have been described as copigments in wines (Boulton, 2001; García-Marino, Escudero-Gilete, Heredia, Escribano-Bailón, & Rivas-Gonzalo, 2013). However, formation of copigmentation complexes not always is a protective factor against anthocyanin disappearance in wines, but depend on the type of copigments and the strength of the interaction established (González-Manzano, Dueñas, Rivas-Gonzalo, Escribano-Bailón, & Santos-Buelga, 2009). Until now the effect of yeasts in copigmentation phenomena is not described however maybe some yeast metabolites could work as copigments.

Color losses during fermentation are mainly due to the change of the polarity of the medium, because of the production of ethanol by yeasts that reduce the solubility of anthocyanins. Later, the polymerization of anthocyanins also can lead to precipitation of the coloring matter. But there is also a significant fraction of anthocyanins that are adsorbed by the yeast cell walls (Morata, Gómez-Cordovés, Colomo, & Suárez-Lepe, 2005), consequently causing a decrease in color of the wine. Nevertheless, this sticking phenomenon is made of weak and reversible interactions (Morata, Gómez-Cordovés, Suberviola, Colomo, & Suárez-Lepe, 2003). What remains unknown is in which extent the adsorbed anthocyanins can be detached from the cell walls throughout the fermentation or aging time.

Regarding yeast contribution to wine color, its ability to synthesize carbonyl compounds along fermentation directly impacts the formation of stable pigments, as they act as precursors of pyranoanthocyanins. Besides, retention of pigments in their cell walls represents a direct loss of color.

As a conclusion, formation of stable pigments during winemaking depends on multiple variables. Usually, polymeric compounds synthesis takes longer than sugar fermentation. In this work, maintaining other variables constant, we focused our research on the influence of the yeast strains used in the synthesis of such desirable compounds.

2. Materials and methods

2.1. Yeast strains assessed and fermentation media

Four *Saccharomyces cerevisiae* yeast strains from Lallemand Inc. (Montreal, Canada), namely Uvaferm™ HPS™, Uvaferm™ VRB™, LALVI™ CLOS™ and 3VA, were studied in this work. Moreover, 7VA (*S. cerevisiae*) and Lalvin™ S6U™ (*S. cerevisiae* × *Saccharomyces bayanus* (formerly *Saccharomyces uvarum*)) were used as control strains. Complementary information about these yeasts can be found as Supplementary material.

For all fermentation assays, except for the evaluation of HCDC activity, a fresh red must of *Vitis vinifera* L. cv Tempranillo (ρ = 1092 at 20 °C; 12.6% v/v PAC; pH 3.2) was used. After crushing the grapes skins and seeds were separated to get a homogenous composition.

All inocula were standardized in order to obtain homogenous active populations (10^6 cfu/ml), by adding 100 µl of each strain to 5 ml of YEPD medium (Kurtzman & Fell, 1998) and growing for 24 h at 23 °C, twice in succession. One ml of inoculum was used to start the fermentation. Fermentations were performed in triplicate using volumes of 70 ml in 100 ml flasks sealed with Müller valves without aeration at 23 °C.

2.2. Determination of HCDC activity

A medium prepared from a concentrated must of *V. vinifera* L. cv Airen was used for hydroxycinnamate decarboxylase (HCDC) activity evaluation. This concentrated must was diluted with distilled water until ρ = 1090 at 20 °C (12.3% v/v Potential alcohol content (PAC)), then *p*-coumaric acid (Fluka, Sigma–Aldrich Corp., Buchs SG, Switzerland) was added at the rate of 50 mg/l and pH was corrected with tartaric acid (Panreac, Barcelona, Spain) to 3.5. Test tubes filled with 10 ml of the fermentation medium were subjected to a heat treatment of 100 °C for 3 min before inoculation. Later, each tube was inoculated with 200 µl of the corresponding yeast strain. The quantity of *p*-coumaric acid after fermentation was determined by high-performance liquid chromatography with photodiode array detection (HPLC–DAD) in an Agilent Technologies (Palo Alto, CA) series 1100 chromatograph, using four concentrations of *p*-coumaric acid as external standard for obtaining the calibration curve (5, 12.5, 25 and 50 mg/l; R^2 = 0.9996). To evaluate degradation of *p*-coumaric acid, yeasts 7VA (HCDC+) and S6U (HCDC−) were used as positive and negative controls respectively.

2.3. Monitoring of acetaldehyde and pyruvic acid synthesis during fermentation

A fermentation assay was carried out in 100 ml volume flasks filled with 80 ml of fresh red must. After soft heat treatment at 100 °C for 3 min, fermentation flasks were inoculated with 1 ml of the corresponding yeast strain. A 2 ml sample was withdrawn every two days during fermentation for acetaldehyde and pyruvic acid analysis. Acetaldehyde concentration was determined by gas chromatography with flame ionization detector (GC–FID) using an Agilent Technologies 6850 equipment (Palo Alto, CA), as described by Morata et al. (2015); while pyruvic acid content was enzymatically analyzed in a Y15 enzymatic autoanalyser (Biosystems, Barcelona, Spain).

2.4. Analysis of anthocyanins adsorbed to yeast cell walls

The ability of adsorbing color molecules by yeasts' cell walls was assessed by two different methods. On the one hand, a qualitative technique based on the visual analysis of growing yeast