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Formation of vinylphenolic pyranoanthocyanins by *Saccharomyces cerevisiae* and *Pichia guillermondii* in red wines produced following different fermentation strategies

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ABSTRACT

The strains of two species of yeast, *Saccharomyces cerevisiae* and *Pichia guillermondii*, both with high hydroxycinnamate decarboxylase (HCDC) activity (56% and 90% respectively), were used in the fermentation of musts enriched with grape anthocyanins, to favour the formation of highly stable vinylphenolic pyranoanthocyanin pigments. The different strains were used to ferment the must separately, simultaneously, or sequentially, the latter involving an initial period using the yeast with the greatest HCDC activity (*P. guillermondii*). The must was made from concentrated grape juice diluted to 220 g/l of sugar, and enriched with grape anthocyanins to 100 mg/l and with *p*-coumaric acid to 120 mg/l. The pH was fixed to 3.5. All 50 ml micro-fermentations were done in triplicate. The development of anthocyanin-3-*O*-glucoside precursors, the decarboxylation of *p*-coumaric acid, and the formation of 4-vinylphenol and vinylphenolic pyranoanthocyanin derivatives were studied during the fermentation. The fermentation strategy used and the yeast HCDC activity significantly influenced the formation of vinylphenolic pyranoanthocyanins. The latter molecules were separated, identified, and quantified using high performance liquid chromatograph with diode array detection and electrospray-mass spectrometry (HPLC-DAD-ESI/MS). The volatile compounds profile was screened during fermentation using gas cromatogrphy-flame ionisation detection (GC-FID), in order to detect and quantify the main molecules. The best results were reached with the sequential fermentation (3.74 mg/l of malvidin-3-*O*-glucoside-4-vinylphenol). This work shows that during mixed or sequential fermentations carried out with non-*Saccharomyces* or highly fermentative *Saccharomyces* strains, with high HCDC activity, the content of stable pigments can be increased without sensorial modifications.

1. Introduction

The traditional role of wine yeasts as transformers of grape sugars into ethanol has been significantly widened since the establishment of modern oenological microbiology. Louis Pasteur (1866) indicated long ago that the types of yeast used in the wineries of a particular region led to that region's wines having similar organoleptic characteristics. Thus, the role of yeasts in winemaking is not purely fermentative. The metabolic activities of a particular wine yeast in a must may lead to the formation of metabolites and to the transformation of grape molecules that may sensorially enrich a wine (Pretorius, 2000). Thus, in recent years, the yeast selection has included the development of techniques for detecting strains that might improve wines in terms of their colour, aroma and structure (Suárez-Lepe & Iñigo, 2004).

Related to colour, some of the metabolites produced by yeasts during the glycolytic stage of fermentation may condense with grape anthocyanins to produce highly stable pyranoanthocyanin adducts, such as vitisin A and B (Fig. 1) (Bakker & Timberlake, 1997; Morata, Calderón, González, Gómez-Cordovés, & Suárez, 2007; Morata, Gómez-Cordovés, Colomo, & Suárez, 2003; Romero & Bakker, 2000). Yeasts with hydroxycinnamate decarboxylase (HCDC) activity can also be used to decarboxylate hydroxycinnamic acids and form vinylphenols that condense with grape anthocyanins to produce vinylphenolic pyranoanthocyanin adducts – molecules that show great colour stability (Fig. 1) (Morata, Gómez-Cordovés, Calderón, & Suárez, 2006; Morata, González, & Suárez-Lepe, 2007). Pigments of this type have been detected in wines using LC–MS techniques, and characterised by mass spectrometry (Hayasaka & Asenstorfer, 2002). With respect to colour improvement, a number of *Saccharomyces cerevisiae* strains show desirable HCDC activity (Shinohara, Kubodera, & Yanagida, 2000), although to very varied degrees. While some show very little activity, others may transform up to 15% of the substrate into

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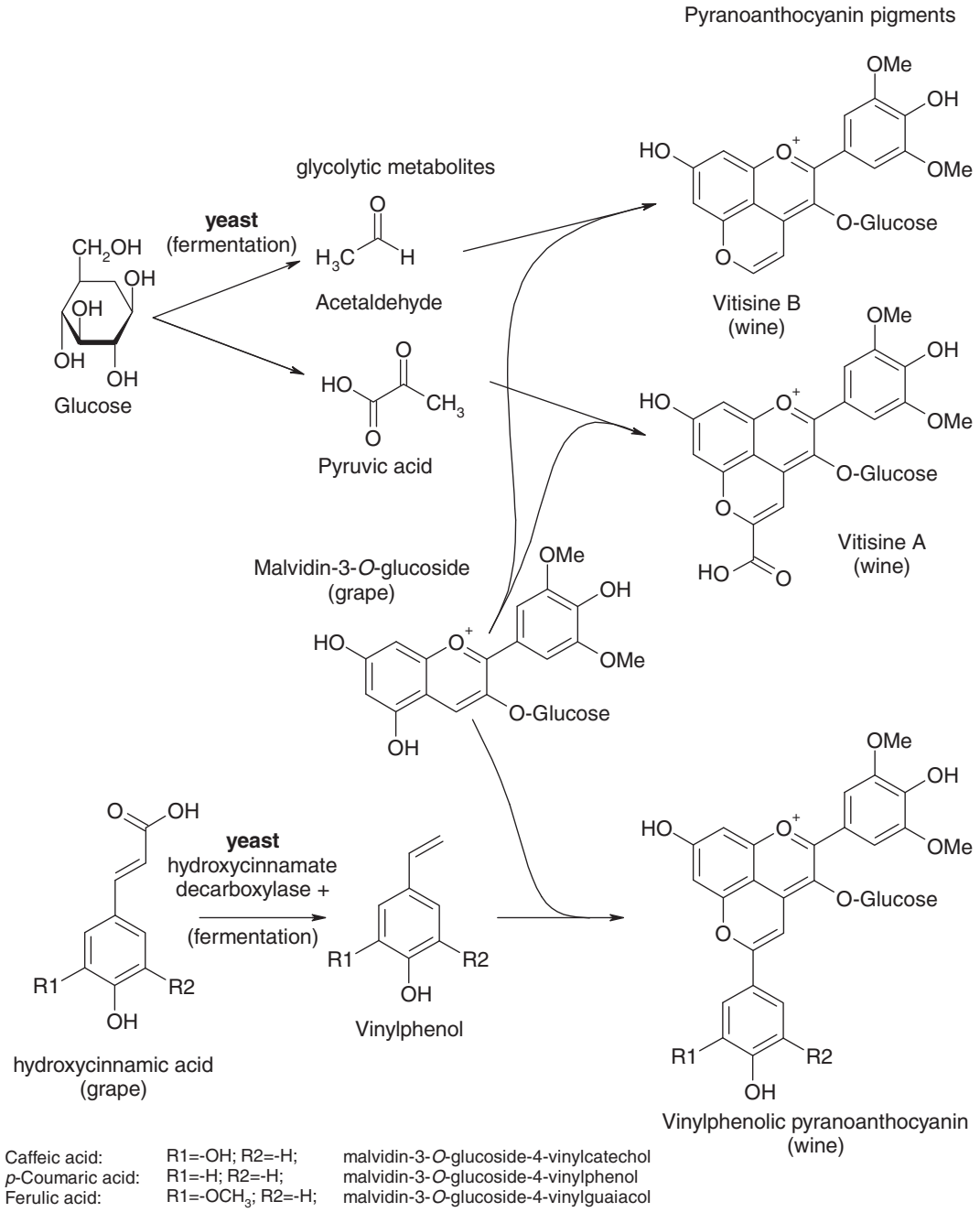

Fig. 1. Formation of pyranoanthocyanins during fermentation using yeasts selected for their strong production of acetaldehyde and pyruvic acid, and/or hydroxycinnamate decarboxylase activity. The derived structures of malvidin-3-*O*-glucoside (quantitatively the most important anthocyanin of *Vitis vinifera* L.) are shown. However, pyranoanthocyanins with analogous structures are also formed from other anthocyanin-3-*O*-glucosides and their acetylic, *p*-coumarilic and caffeoilic derivatives.

vinylphenols (Benito, Palomero, Morata, Calderón, & Suárez-Lepe, 2009). Vanbeneden, Gils, Delvaux, and Delvaux (2008) showed that of the 75 *S. cerevisiae* strains they examined, over 70% were capable of decarboxylating ferulic acid. However, only a few *Saccharomyces* strains showed high conversion rates.

HCDC activity has been described in several yeast, bacterial and fungal species (Chatonnet, Boidron, & Dubordieu, 1993; Degrassi, Laureto, & Bruschi, 1995; Dias et al., 2003; Edlin, Narbad, Gasson, Dickinson, & Lloyd, 1998; Suezawa, 1995; Suezawa, Yoshioka, & Mori, 1998). Some species, such as *Pichia guillermondii* show high HCDC activity. However, *P. guillermondii* is unable to completely ferment a normal must with a sugar content of 220 g/l, as its normal fermentation limit is 30 g/l; therefore it can only be used alongside *S. cerevisiae* (high fermentative power) in a mixed or sequential culture for wine fermentation to be completed. A mixed culture would involve inoculating the must with a mixture of *P. guillermondii* and *S. cerevisiae*; a sequential culture would require initial inoculation with *P. guillermondii* to favour the formation of vinylphenols, followed by inoculation with *S. cerevisiae* to ensure the fermentation is properly completed.

In this work, the HCDC activity of different wine *Saccharomyces* strains was compared with a non-*Saccharomyces* species, *P. guillermondii*, with the latter showing the highest activity. Therefore, this works aim was to use this non-*Saccharomyces* yeast either together (mixed fermentation) or following (sequential fermentation) the *Saccharomyces* strain, in order to increase the amount of