SENSORIAL REPERCUSSIONS OF THE FORMATION OF VINYLPHENOLIC PYRANOANTHOCYANINS

Suárez-Lepe, J. A.[(1)]; Morata, A.[(1)]; Benito, S.[(1)]; Palomero, F.[(1)]; Hernández, M. T.[(2)]
(1) Dept. Tecnología de Alimentos. E. T. S. Ingenieros Agrónomos. Universidad Politécnica de Madrid. Madrid. 28040. España.
(2) IFI. CSIC
Tlf. 00 34 913365730
E-mail: antonio.morata@upm.es.

RESUMEN

Utilizando cepas de levaduras seleccionadas de la especie *Saccharomyces cerevisiae* con actividad hidroxicinamato descarboxilasa (HCDC+) se puede favorecer la transformación de los antocianidin-3-O-glucosidos de la uva en piranoantocianos vinilfenólicos. La selección clonal con adecuadas propiedades metabólicas y fermentativas para la elaboración de vinos tintos, permite así estabilizar la materia colorante gracias a la formación de piranoantocianos. La eliminación de parte del contenido de ácidos hidroxicinámicos de la uva permite reducir las posibilidades de formación de etilfenoles en vinos tintos destinados a crianza y contaminados en barrica con levaduras de los géneros *Brettanomyces*/*Dekkera*. En ensayos piloto se puede reducir notablemente la formación de etilfenoles partiendo de contenidos iniciales de 12 mg l^{-1} de ácido *p*-cumárico, llegándose a concentraciones por debajo del límite de detección.

ABSTRACT

Using selected yeast, of the *Saccharomyces cerevisiae* specie, with hydroxycinnamate decarboxylase activity (HCDC+) we can transform anthocyanidin-3-O-glucosides from grapes into vinylphenolic pyranoanthocyanins. *Saccaromyces* cerevisiae don't posses vinylphenol reductase activity (VPhR), however some yeast strains belonging to this specie are HCDC+. The selection of *Saccharomyces cerevisiae* strains HCDC+ with suitable fermentative and metabolic properties to red wine making allow us to stabilize the wine anthocyanins forming resistant pyranoanthocyanins. A relevant repercussion is that removing of hydroxycinnamic acids from musts we can avoid the formation of ethylphenols in red wine contaminated with the spoiling yeast *Dekkera*/*Brettanomyces* during barrel ageing. During in-vitro experiments we reduce notably the initial concentrations of 12 mg l^{-1} of *p*-coumaric acid, allowing can be below the detection limit in many vinifications.

INTRODUCTION

Selected yeast, of the *Saccharomyces cerevisiae* specie, with hydroxycinnamate decarboxylase activity (HCDC+) can transform anthocyanidin-3-O-glucosides from grapes into vinylphenolic

pyranoanthocyanins. *Saccaromyces* cerevisiae don't posses vinylphenol reductase activity (VPhR), however some yeast strains belonging to this specie are HCDC+. The selection of *Saccharomyces cerevisiae* strains HCDC+ with suitable fermentative and metabolic properties to red wine making allow us to stabilize the wine anthocyanins forming resistant pyranoanthocyanins (**Figure 1**; Morata et al. 2006 and 2007).

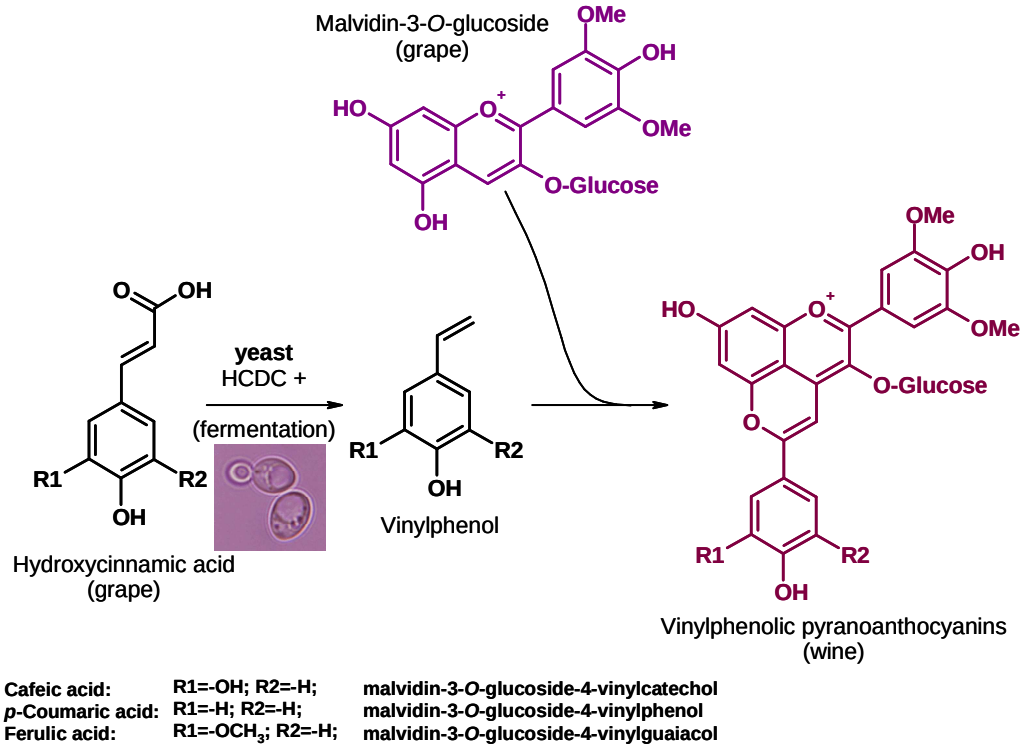

Figure 1. Vinylphenolic pyranoanthocyanin formation using HCDC+ yeast strains of Sacch. cerevisiae during fermentation

Pyranoanthocyanins are very stable pigments as they have a double heteroaromatic ring owing more resonant forms than other anthocyanins which allow a high stability against oxidative damage, hydrolysis reactions and colour modifications by pH variations. Moreover, pyranoanthocyanins are resistant to SO_2 bleaching; due to their saturated electrophilic C4 position which after attacks HSO_3^- (**Figure 2**).

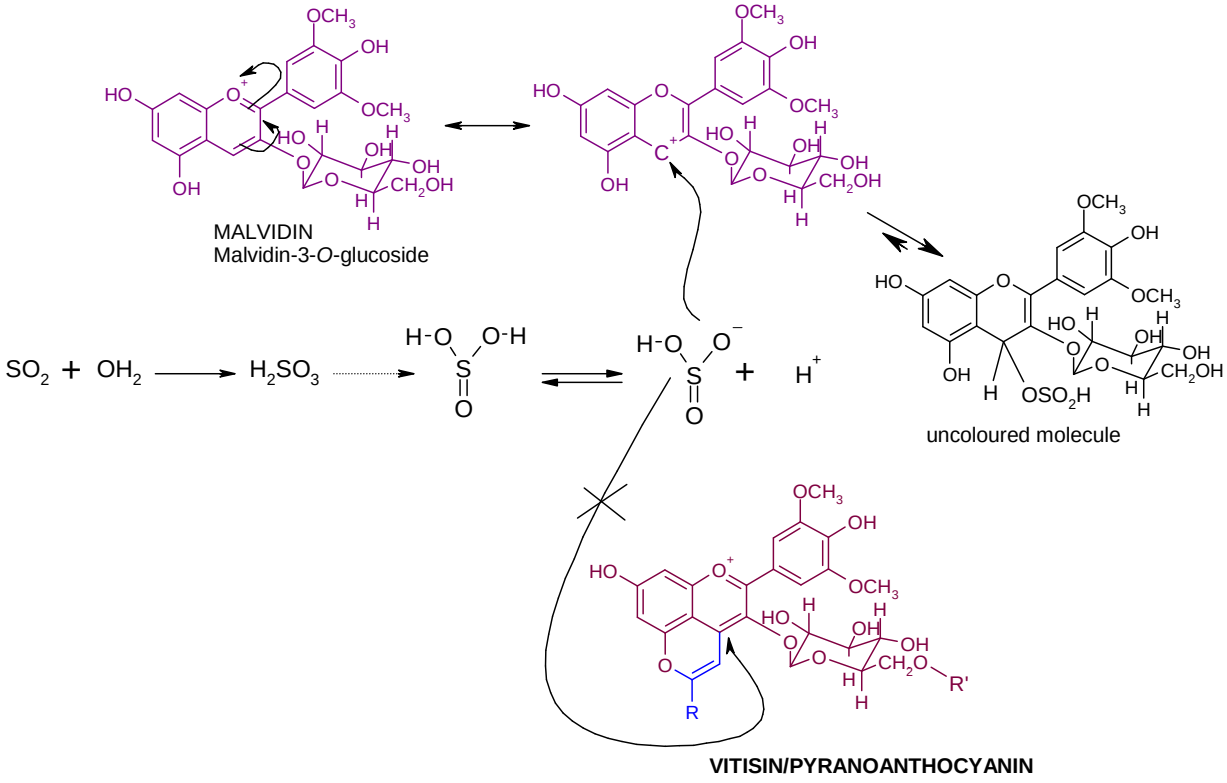

Figure 2. Inhibition of SO_2 bleaching in vinylphenolic pyranoanthocyanins

MATHERIALS AND METHODS

Fermentations

Red must from *Vitis vinifera* cv Tempranillo were fermentated usig diferent strains of *Saccharomyces* with and without HCDC activity enriched with *p*-coumaric and ferulic acid in order to study the vinylphenolic pyranoanthocyanins formation during fermentation.

The reduction of the initial content of p-coumaric acid was measured by GC-MS.

Anthocyanin evolution during fermentation

Anthocyanin contents evolution and vinylphenolic pyranoanthocyanin formation was monitored using RP-HPLC-DAD-ESI/MS. The separation was performed using a gradient of two eluyents (Formic acid:MQ water 10:90 and Formic acid:Methanol) in an RP Novapack C18 column (Waters).

Malvidin-3-O-glucoside, p-coumaric acid and 4-ethylphenol used as standards were from extrasynthese (Genay, France)

RESULTS AND DISCUSSION

Vinylphenolic pyranoanthocyanin formation using HCDC+ yeast strains of *Sacch. cerevisiae*

In wines from grapes with appropriate contents of hydroxycinnamic acids we can form high amounts of pyranoanthocyanins using selected yeast strains HCDC+. The formation of malvidin-3-O-glucoside-4-vinylguaiacol from ferulic acid is especially interesting, because of the colour intensity/tonality reached in the wine and for the great amount synthesized (**Figure 3**).

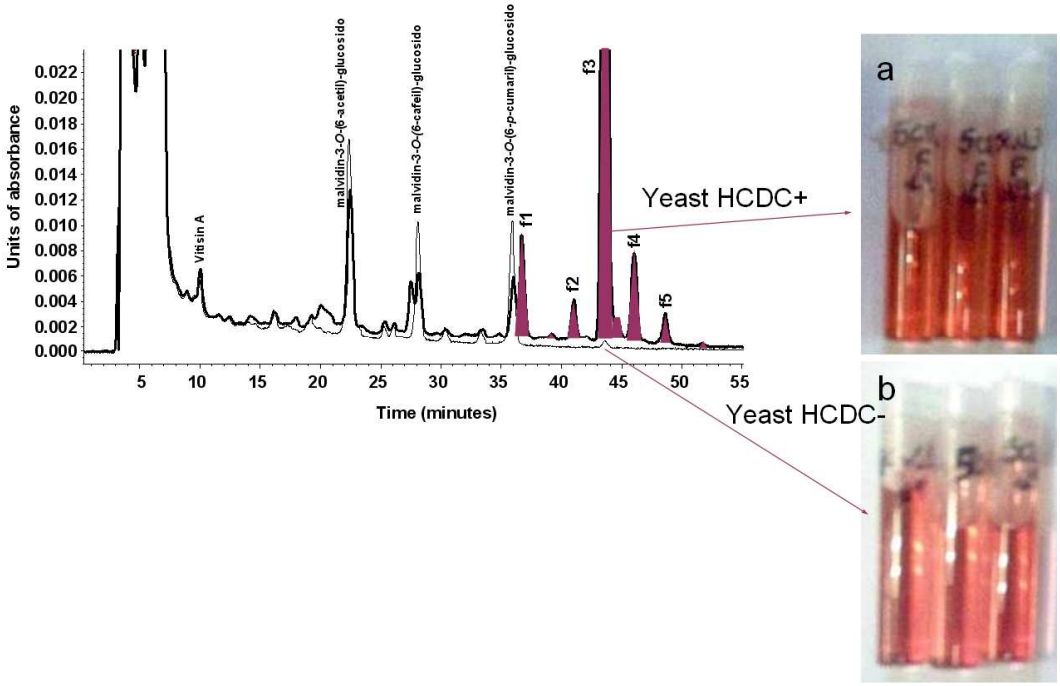

Figure 3. Fermentations performed in triplicate using both a yeast strain HCDC+ (a) and another HCDC- (b) with ferulic acid addition

Formation of vinylphenolic pyranoanthocyanins during fermentation let reduce ethylphenols precursors in wines. Protection against *Brettanomyces*.

Vinylphenolic pyranoanthocyanin formation led us to remove hydroxycinnamic acids from musts avoiding the presence of ethylphenol precursors (Suárez et al, 2007), therefore we can naturally and without additions stabilize red wines against *Brettanomyces* (**Figura 5**).

A relevant application of HCDC+ *Sacch. cerevisiae* yeast strains is that removing hydroxycinnamic acids from musts we can avoid the formation of ethylphenols in red wine contaminated with the spoiling yeast *Dekkera/Brettanomyces* during barrel ageing. During in-vitro experiments we reduce the ethylphenol formation more than 3 times from initial concentrations of 12 mg l^{-1} of *p*-coumaric acid. This result let us extrapolate that in grape

varieties with lower p-coumaric contents we can reduce the precursor hydroxycinnamic acid to a quantity that transformed totally in ethylpehnol will be below the detection limit.

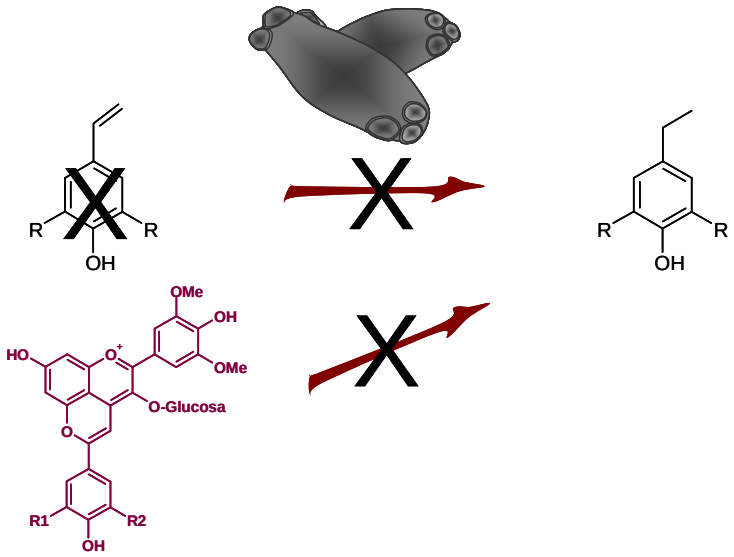

Figure 5. No ethylphenol formation after fermentation with *Sacch. cerevisiae* HCDC+.

Moreover, we have demonstrated the imposibility to release vinylphnols from vinylfenolic pyranoanthocyanins in presence of *Brettanomyces*, because these yeast are unable to break the pigment and therefore are not capable to reduce the vinylphenol moiety to ethylphenol. This pigments are a sure and natural way to preserve red wine color and to remove the potential precursors to ethylphenol formation.

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