Reduction of 4-ethylphenol contents in red wines using HCDC+ yeasts and cinnamyl esterases.

Antonio MORATA, Ricardo VEJARANO, Santiago BENITO, Felipe PALOMERO, Carmen GONZALEZ, Wendu TESFAYE, Carlos UTHURRY, José Antonio SUÁREZ-LEPE
enotecUPM

Departamento de Tecnología de Alimentos - Universidad Politécnica de Madrid
Ciudad Universitaria, S/N, 28040 Madrid
Email: antonio.morata@upm.es
Telf: 0034 91 336 57 45

Introduction

4-Ethylphenol (4-EP) is a recurrent problem at red wine cellars produced by the spoilage yeast *Brettanomyces* (*Dekkera*). Several techniques have been published either to control *Brettanomyces* or to remove 4-EP usually with unsuitable results (Suárez et al., 2007).

Hydroxycinnamate decarboxylase (HCDC) activity is frequent in many wine yeast species. HCDC+ yeasts decarboxylate hydroxycinnamic acids (HCAs) producing vinyl phenols (VPs). VPs can spontaneously condense with grape anthocyanins during fermentation yielding vinylphenolic pyranoanthocyanins (VPAs; Morata et al., 2007). These compounds are very stable pigments in enological conditions.

HCDC+ yeasts used in the fermentation of red grapes increase the formation of VPAs (Suárez-Lepe y Morata, 2012). *Brettanomyces* is not able to use the VP moiety integrated in VPAs to form EPs. Therefore this technique is a natural way both to reduce the precursors of 4EP and to increase the formation of stable pigments (Benito et al, 2009). In grapes the total pool of HCAs is formed by free HCAs but also by tartaric esters of HCAs (TE-HCAs). These compounds are in higher amounts than free HCAs, being a reservoir, which can release slowly HCAs during barrel ageing by acidic hydrolysis , hence, they can be available for *Brettanomyces* to produce EPs.

Cinnamyl esterases (CEs) are enzymes able to hydrolyze TE-HCAs releasing free HCAs. The combined use of both CEs and HCDC+ yeast strains is a **natural enzymatic-biological-chemical way** to reduce the formation of EP by transformation of their precursors (VPs) in stable pigments (Figure 1; Morata et al., 2013).

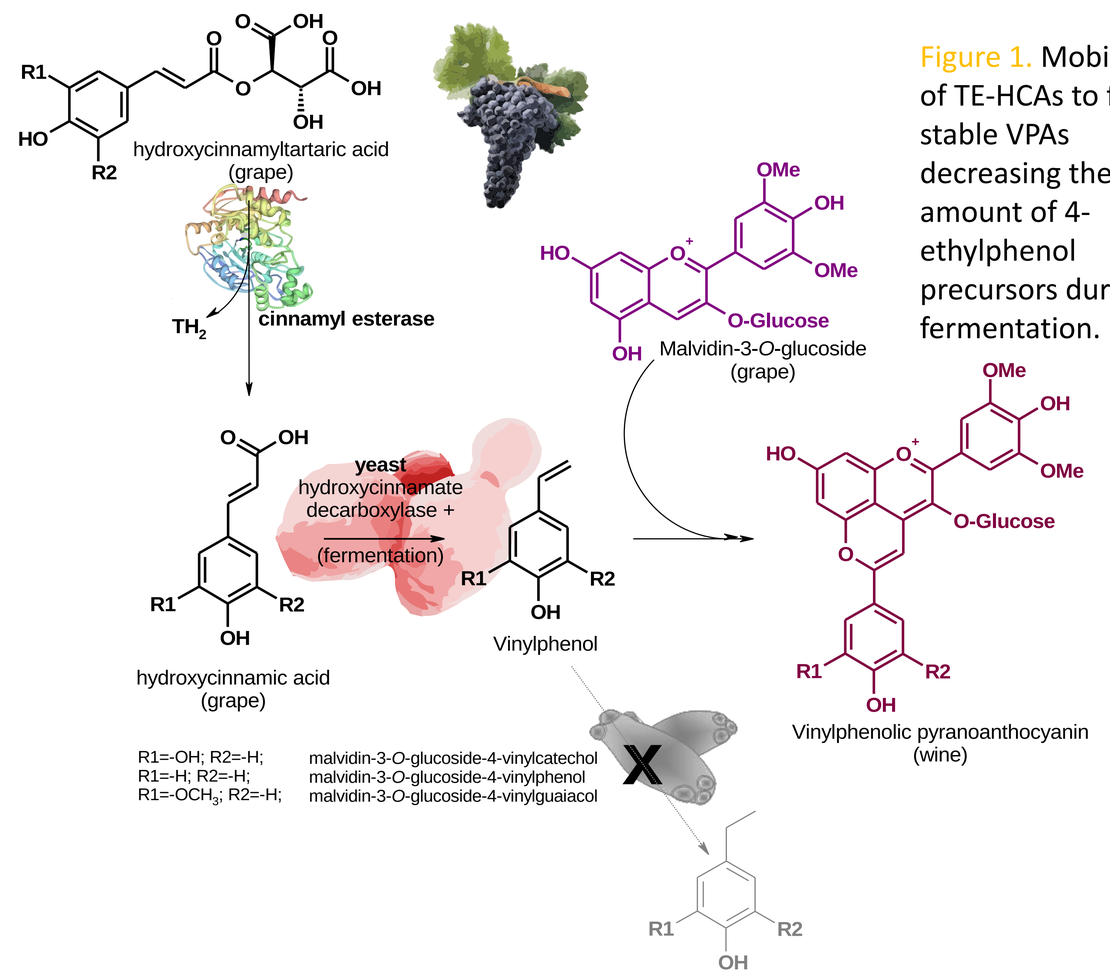

Figure 1. Mobilization of TE-HCAs to form stable VPAs decreasing the amount of 4-ethylphenol precursors during fermentation.

Results and Discussion

The HCDC activity of 17 commercial and experimental yeasts ranged between 35 and 99%. The yeast S6U (*S. uvarum*) shows absence of HCDC activity and was used as control.

The production of VPAs by yeasts strains with HCDC activity in fermentations of musts has been variable ranging from non-detection (strain S6U , HCDC−) to 0.9 mg/l (strain 7VA, HCDC+) (Figure 2). When the must was supplemented with CEs the amount of VPAs increased considerably (1.1–3.3 mg/l) due to the release of HCAs from their corresponding TEs. When CEs were used in fermentations with HCDC− yeasts the formation of VPAs was non-detected as can be observed for strain S6U.

The final concentration of EPs after *Dekkera* development in fermentations that had been added of CEs the values were generally higher than in controls without enzymes but quite acceptable when HCDC+ strains were used (range 22–682 µg/l). Moreover the fermentations with 7 yeast strains showed values **below or close to 400 µg/l** (the sensory threshold for 4EP) (Figure 3). However, when CEs were used together with HCDC- there was no formation of VPAs during fermentation, and after *Dekkera* contamination the levels of 4-EP were around 1150 µg/l (3-folds sensory threshold for 4EP). This situation shows the acceleration of the normal process. TE-HCAs are slowly hydrolyzed during barrel ageing and when *Dekkera*/*Brettanomyces* contaminates the wine, 4-EP levels increase dramatically.

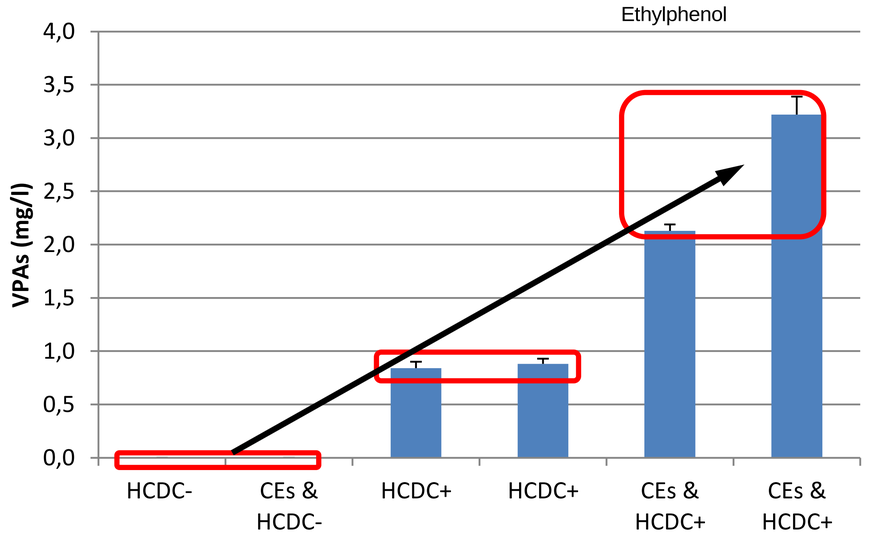

Figure 2. Formation of stable pigments from grape anthocyanins (VPAs) during fermentation with and without CEs. Yeasts HCDC- S6U (*S. uvarum*); Yeasts HCDC+ 7VA & TP2A16 (*S. cerevisiae*).

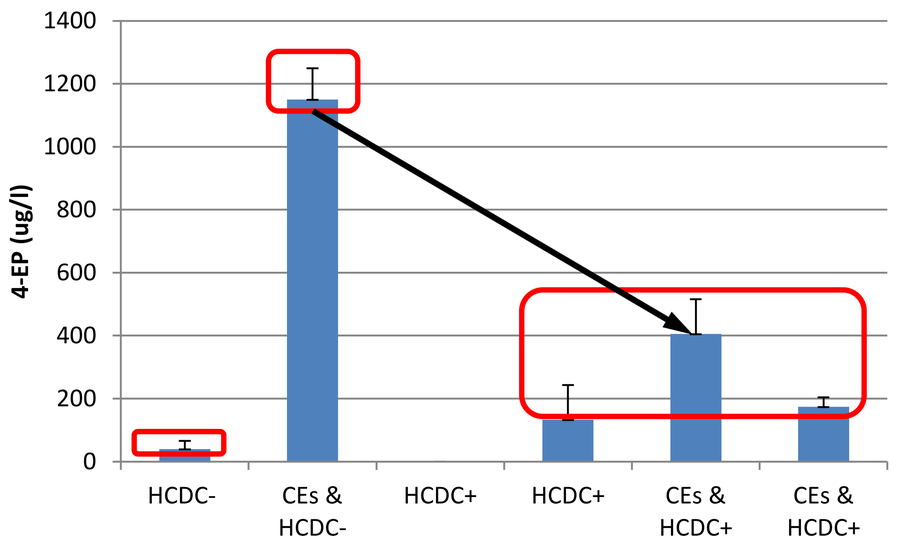

Figure 3. Formation of 4-EP after *Dekkera* contamination (10^6 cfu/ml) in wines from musts treated with and without CEs and fermented by yeasts HCDC- S6U (*S. uvarum*) and HCDC+ 7VA & TP2A16 (*S. cerevisiae*).

Conclusions

The use of both CEs and HCDC+ yeast strains is a **natural enzymatic-biological-chemical way to reduce the formation of EPs precursors** (VPs) blocking them in stable pigments and avoiding the formation of high amounts of 4-EP if wine is contaminated by *Brettanomyces* during barrel ageing

Materials and Methods

The HCDC activity of 17 commercial and experimental yeasts has been analyzed by fermentation in YEPD media with p-coumaric acid. The degradation of p-coumaric acid was measured by HPLC-DAD.
Red must treated with 30 mg/l CEs (Rapidase Maxifruit, DSM Food Specialties B.V. Delft,The Netherlands) was fermented in triplicate using HCDC+ yeasts isothermally at 25 ºC. Controls without addition of CEs and also using HCDC- were used. After fermentation the wines were contaminated with 10^6 cfu/ml of *Dekkera* D37 to evaluate the formation of EPs.
Anthocyanins and pyranoanthocyanins were analyzed by HPLC-DAD-ESI/MS according to Morata et al., 2007. Ethylphenols and vinylphenols were analyzed by GC-MS in SIM mode after liquid extraction with dichloromethane according to Morata et al., 2013.

References

Morata A, Gómez-Cordovés MC, Calderón F, Suárez JA. Effects of pH, temperature and SO2 on the formation of pyranoanthocyanins during red wine fermentation with two species of *Saccharomyces*. International Journal of Food Microbiology, 2006,106, 123-9.
Benito S, Palomero F, Morata A, Uthurry C, Suárez-Lepe JA. Minimization of ethylphenol precursors in red wines via the formation of pyranoanthocyanins by selected yeasts. International Journal of Food Microbiology, 2009, 132, 145-52.
Morata A, González MC, Suárez JA. Formation of vinylphenolic pyranoanthocyanins by selected yeasts fermenting red grape musts supplemented with hydroxycinnamic acids. International Journal of Food Microbiology, 2007, 116, 144-52.
Suárez R, Suárez-Lepe JA, Morata A, Calderón F. The production of ethylphenols in wine by yeasts of the genera *Brettanomyces* and *Dekkera*: a review. Food Chemistry 2007, 102, 10-21.
Suárez-Lepe JA, Morata A. New trends in yeast selection for winemaking. Trends in Food Science & Technology, 2012, 23, 39-50.
Morata A, Vejarano R, Ridolfi G, Benito S, Palomero F, Uthurry C, Tesfaye W, González C, Suárez-Lepe JA. Reduction of 4-ethylphenol production in red wines using HCDC+ yeasts and cinnamyl esterases. Enzyme and Microbial Technology, 2013, 52, 99-104.

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