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).

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 TE. 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 7VA 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.

Conclusions

The use of both CEs and HCDC+ yeast strains is a natural enzymatic-biological-chemical way to reduce the formation of EP 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 analysed 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 cg/ml CEs (Rapaidae Manzfrud, DSM Food Specialties B.V. Dein, The Netherlands) was fermented in triplicate using HCDC+ yeasts isothermally at 25 °C. Controls without addition of CEs and also using HCDC− were used. After fermentation the wines were contaminated with 10°/50°/55° of Dekkera 237/977 to evaluate the formation of EPs.

Anthocyanins and pyranoanthocyanins were analysed by HPLC-DAD-ESI/MS according to Morata et al., 2007. Ethylphenols and vinylphenyls were analysed by GC-MS in SIM mode after liquid extraction with dichloromethane according to Morata et al., 2013.

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