Use of *Schizosaccharomyces pombe* to produce stable pigments during red winemaking

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INTRODUCTION

The study of microbiological technologies that help better preserve the color of wine during prolonged ageing is essential to ensure the quality and longevity of the final product. Pyranoanthocyanin pigments are a family of anthocyanin derivatives with a double pyrano structure that provides them with more stability under oxidizing conditions and greater resistance to wine decoloration due to SO_2 bleaching effect (Bakker *et al.*, 1997; Romero and Bakker, 1999; Romero and Bakker, 2000). The formation of these pyranoanthocyanins pigments can be increased during fermentation using appropriate yeasts able to produce and release their precursors in the fermentative media (Fig. 1). *Schizosaccharomyces pombe* is a non-*Saccharomyces* yeast very interesting due to its peculiar metabolism that can increase the formation of stable pigments through the release of vitisin precursors or through its hydroxycinnamate decarboxylase activity (Suárez-Lepe *et al.*, 2012).

MATERIAL AND METHODS

S. pombe yeast strains assessed were 935, 936, 938 and 2139 (CSIC, Madrid, Spain). *S. cerevisiae* 7VA (HCDC+) (EnotechUPM, Madrid, Spain) and *S. uvarum* S6U (HCDC -) (Lallemand, Montreal, Canada) were used as control strains.

Anthocyanins (HPLC-DAD/MS), acetaldehyde (GC-FID) and pyruvic acid (enzymatic tests) analysis were performed periodically to track their evolution. While glucose, fructose, malic acid (all by enzymatic tests) and colour (spectrophotometry) were only analyzed at the end of the fermentation (day 28).

RESULTS AND DISCUSSION

Total pyranoanthocyanins ranged from 11.9 to 19.4 mg/l depending on the strain of *S. pombe* used.

S. pombe 938 strongly produce vitisin A (more than 10 mg/l) and acetylvitisin A (more than 3 mg/l) compared with *S. cerevisiae* 7VA selected for its good pyruvic acid production.

Vinylphenolic pyranoanthocyanins maximum production was <1.5 mg/l (strain 2139), so its influence on colour would only be important in aged red wines as the grape anthocyanins begin to disappear (Fig. 2).

The wine produced by *S. pombe* 938 showed a more intense colour (CI: 0.81 ± 0.01) than those produced by the other *Schizosaccharomyces* strains and *Saccharomyces* species, since it had the highest total pyranoanthocyanins content (19.4 mg/l).

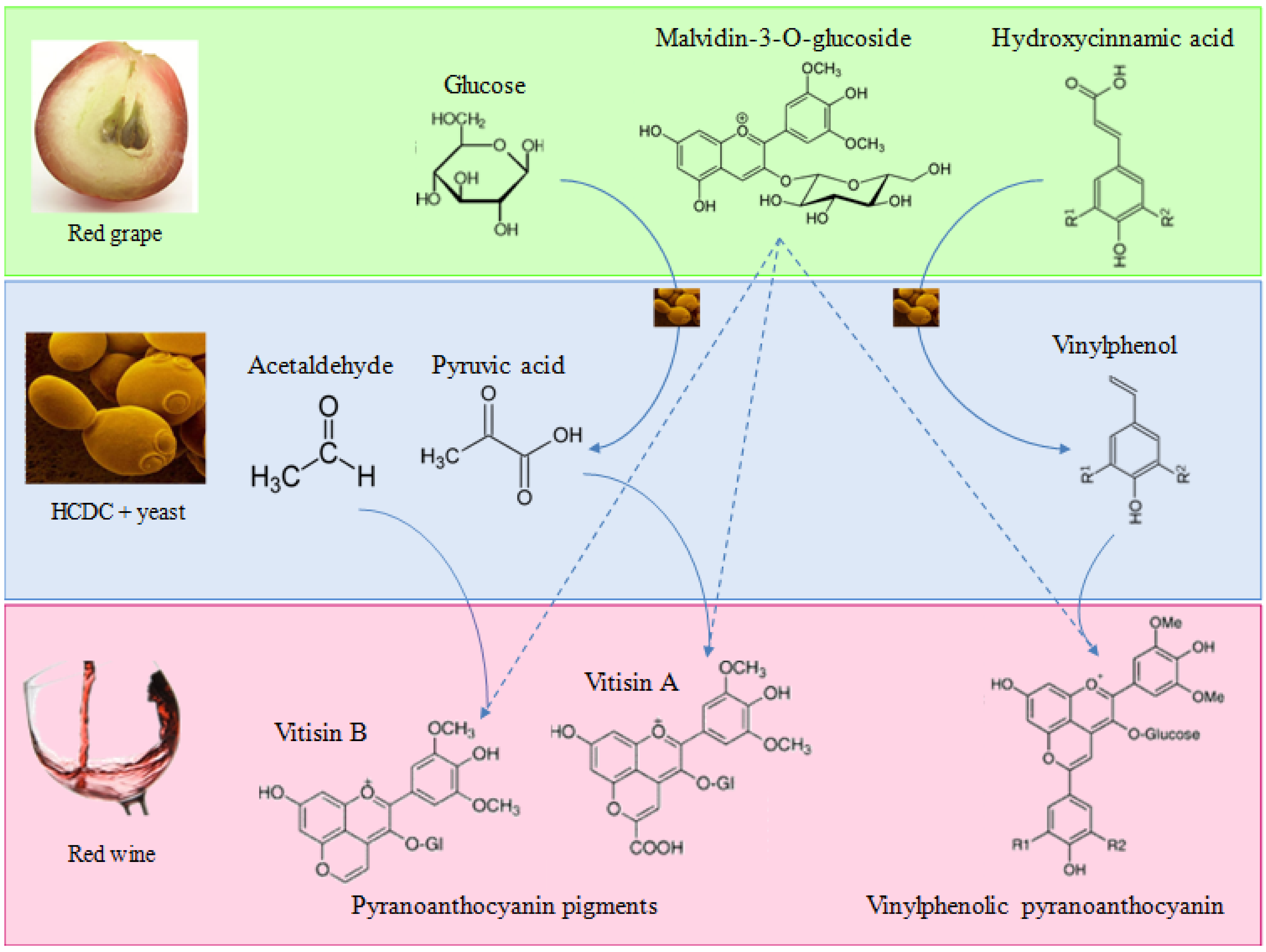

Fig. 1. Formation of pyranoanthocyanins during fermentation using yeasts selected for their strong production of acetaldehyde and pyruvic acid, and/or hydroxycinnamate decarboxylase activity.

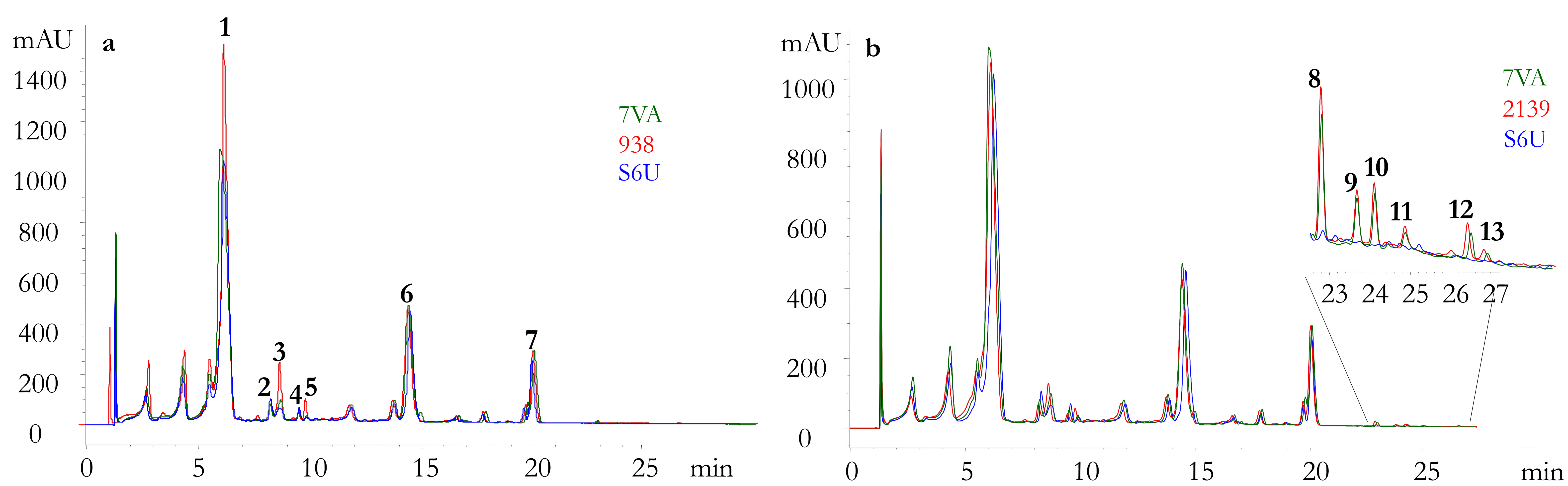

Fig. 2. Chromatographic representations of the major pyranoanthocyanins produced during fermentation of red must. 1: malvidin-3-O-glucoside; 2: Vitisin B; 3: Vitisn A; 4: acetylvitisin B; 5: acetylvitisin A; 6: malvidin-3-O-(6''-acetylglucoside); 7: malvidin-3-O-(6''-p-coumaroylglucoside); 8: malvidin-3-O-glucoside-4-vinylphenol; 9: malvidin-3-O-glucoside-4-vinylguaiacol; 10: malvidin-3-O-(6''-acetylglucoside)-4-vinylphenol; 11: malvidin-3-O-(6''-acetylglucoside)-4-vinylguaiacol; 12: malvidin-3-O-(6''-p-coumaroylglucoside)-4-vinylphenol; 13: malvidin-3-O-(6''-p-coumaroylglucoside)-4-vinylguaiacol. HPLC-DAD/MS λmax = 525

CONCLUSIONS

The use of *S. pombe* to increase the formation of pyranoanthocyanins provides new possibilities for improving the stability of wine pigments and, therefore, wine colour. The selection of *S. pombe* strains releasing larger amounts of pyruvic acid and/or having better hydroxycinnamate decarboxylase activity, should improve pyranoanthocyanin pigment production.

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