

PRODUCTION OF VOLATILE PHENOLS BY WINE PEDIOCOCCI

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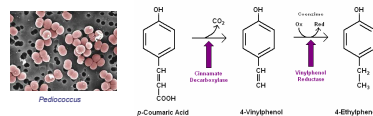
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ABSTRACT

Some wine microorganisms can produce volatile phenols (vinylphenols and ethylphenols) from grape-derived hydroxycinnamic acids (*p*-coumaric and ferulic acids). Volatile phenols have characteristic aromas which, above a certain concentration threshold, have a negative effect on the overall aroma of a wine, but at low concentrations have been cited as contributing positively to aroma complexity. The contaminant yeasts *Dekkera/Brettanomyces* are recognized as the main volatile phenols producer organisms, although previous works have shown that some strains of Lactic Acid Bacteria (LAB) are also capable of producing volatile phenols under wine conditions. In this work, it is shown that wine pediococci have the ability to produce volatile phenols from the corresponding phenolic acids. Cells cultivated in liquid media supplemented with *p*-coumaric and ferulic acids, produced volatile phenols in the growth media, which were analysed by GC-FID. The results indicate the existence of strain variability and a much higher capacity to produce vinylphenols (4-vinylphenol and 4-vinylguaiacol, the intermediate compounds of the hydroxycinnamic acid metabolic pathway) than ethylphenols. Higher conversion yields were found for *p*-coumaric acid than for ferulic acid. The higher the *p*-coumaric acid concentration the higher the 4-vinylphenol concentration obtained. However, in terms of molar conversion yield, a maximum was reached at around 1.5 M (250 ppm) of substrate, stabilising at higher values. The production of volatile phenols was found to be concomitant with the growth curves of the bacteria. This study shows that wine pediococci have hydroxycinnamic acid decarboxylase activity, thus being able to produce vinylphenols from phenolic acids in sensorially significant amounts.

INTRODUCTION

- Volatile phenols are aromatic compounds that affect, in a negative way, the wine quality giving "animal", "leather", "horse sweat" odors to the wine. The presence of these compounds in wine is nowadays of great concern among wine producers being considered a key point in the control of wine quality.
- The precursors of the volatile phenols are natural constituents of grape juice and wine – the hydroxycinnamic acids *p*-coumaric and ferulic acids. The transformation involves a sequence of two enzymatic activities. In the first, a carboxylase decarboxylates the hydroxycinnamic acid in the corresponding vinyl derivative (4-vinylphenol from *p*-coumaric acid or 4-vinylguaiacol from ferulic acid). In the second reaction a reductase converts the vinyl in the corresponding ethyl compound (4-ethylphenol or 4-ethylguaiacol) (FIGURE 1).
- Which microorganisms are involved in the metabolism of the hydroxycinnamic acids has been under discussion. The ability of *Brettanomyces* to produce volatile phenols has been well demonstrated in the literature (Heresztyn, 1986; Chatonnet *et al.* 1995; Edlin *et al.* 1995) being probably the main organism involved.



- Other yeasts and bacteria have, however, been shown to be able to metabolize hydroxycinnamic acids (Edlin *et al.* 1995). Concerning bacteria, Cavin *et al.* (1993) have shown that *p*-coumaric and ferulic acids were decarboxylated by *Lactobacillus brevis*, *Lact. plantarum* and *Pediococcus*. Chatonnet *et al.* (1995) reported that some strains of *Lact. brevis* and *Ped. pentosaceus* were capable of decarboxylating *p*-coumaric acid to form 4-vinylphenol as actively as *S. cerevisiae*. *Oenococcus oeni* synthesized very small quantities of 4-vinylphenol. Only *Lact. plantarum* was capable of producing ethylphenols, but in low concentrations when compared to *Brettanomyces*. More recently, Couto *et al.* (2006) screened 35 LAB strains (20 species) for the ability to produce volatile phenols. 37% of these strains were found to be able to produce volatile phenols from *p*-coumaric acid.
- Thus, it can be seen in the literature, that certain LAB, pediococci strains included, decarboxylate phenolic acids into vinylphenol; the capacity then to produce ethylphenols being much rarer.
- Aside from standard media favorable to their development, the ability of LAB to produce volatile phenols in wine was never studied in depth. Further research is needed concerning the role of wine microorganisms (other than *Brettanomyces*), and possible interactions between them, in the production of volatile phenols.

The main objective of this work:

To characterise the ability of wine pediococci to produce volatile phenols from the corresponding phenolic acids

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RESULTS

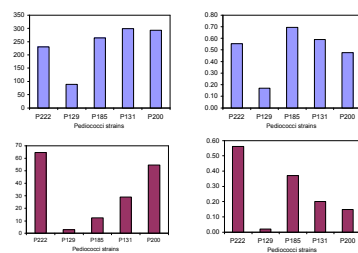


FIGURE 2 - Production of vinylphenol and ethylphenol from 500 ppm of *p*-coumaric acid (Blue bars) and vinylguaiacol and ethylguaiacol from 500 ppm of ferulic acid (Bordeaux bars)

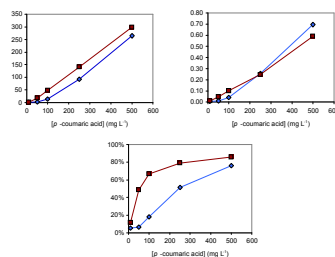


FIGURE 3 - Production of vinylphenol and ethylphenol from different concentrations of *p*-coumaric acid. *Pediococcus* sp ESB185 (Blue lines) and *Pediococcus pentosaceus* NCFB990 (Bordeaux lines)

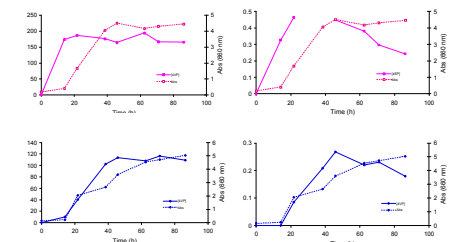


FIGURE 4 - Production of volatile phenols from 500 ppm of *p*-coumaric acid during the growth of *Pediococcus pentosaceus* NCFB990 (above) and *Pediococcus* sp ESB185 (below) in MRS/TJ medium

MATERIALS AND METHODS

Bacterial strains

TABLE 1 - List of strains used in this study

Strains	
P185	<i>Pediococcus</i> sp ESB185
P200	<i>Pediococcus</i> sp ESB200
P222	<i>Pediococcus</i> sp ESB222
P129	<i>Pediococcus damnosus</i> NCFB1832
P131	<i>Pediococcus pentosaceus</i> NCFB990

ESB – Escola Superior de Biotecnologia Collection, Porto, Portugal
NCFB – National Collection of Food Bacteria, Reading, England

Growth conditions and production of volatile phenols in synthetic medium

Cultures were grown aerobically at 25°C, without agitation, to late exponential phase in MRS/TJ, pH 4.5, containing hydroxycinnamic acids at 500, 50 or 5 mg/L *p*-coumaric (*p*-hydroxycinnamic) acid, ferulic (*p*-hydroxymethoxycinnamic) were obtained from Sigma-Aldrich (Steinheim, Germany).

Analysis of volatile phenols

Samples (50 mL) were taken at late exponential growth phase (5-7 days) from culture media and were centrifuged at 3000 x g for 10 min. Volatile phenols in the culture supernatants were analysed by GC-FID according to the method developed by Bertrand (1981).

RESULTS / CONCLUSIONS

- The 5 pediococci strains studied were found to produce volatile phenols from the metabolism of the hydroxycinnamic acids: *p*-coumaric and ferulic acids (FIGURE 2). Strain variability in terms of the capacity to produce volatile phenols is observed
- The capacity to produce volatile phenols from ferulic acid is lower than from *p*-coumaric acid (FIGURE 2)
- The production of vinyl compounds is higher than the production of ethyl compounds (FIGURE 2). This observation evidences a high decarboxylation activity of the precursor (phenolic acid) and a low reduction activity of the intermediate compound (see pathway on FIGURE 1)
- The higher the *p*-coumaric acid concentration the higher the 4-vinylphenol concentration obtained. However, in terms of molar conversion yield, a maximum was reached at around 1.5 M (250 ppm) of substrate, stabilising at higher values (FIGURE 3)
- The production of volatile phenols was found to be concomitant with the growth curves of the bacteria specially for strain *Pediococcus* sp. ESB185. Strain *Pediococcus pentosaceus* NCFB990 produces volatile phenols before the detection of the onset of the exponential growth phase (FIGURE 4)
- This study shows that wine pediococci have hydroxycinnamic acid decarboxylase activity, thus being able to produce vinylphenols from phenolic acids in sensorially significant amounts. The reductase activity was found to be is much lower

Future steps

- To evaluate the capacity of the bacteria to produce volatile phenols in wine or near wine conditions
- To study the factors which may influence the production of volatile phenols

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