The influence of wine phenolic acids on the production of Volatile Phenols by Lactic Acid Bacteria

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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. In this work, the effect of hydroxybenzoic and hydroxycinnamic acids on the production of volatile phenols from *p*-coumaric acid by some strains of LAB was studied. Cultures in late exponential phase were grown in MRS medium with 50 mg l^{-1} *p*-coumaric acid, supplemented with different phenolic acids. It was found that the hydroxycinnamic acids tested (caffeic and ferrulic) clearly stimulate the production of volatile phenols from the metabolism of p-coumaric acid, while not affecting bacterial growth. The hydroxybenzoic acids studied (gallic and vanillic) did not significantly affect the capacity of LAB to synthesize volatile phenols. Results suggest that hydroxycinnamic acids induce the biosynthesis of enzymes involved in the metabolic pathway: cinnamate decarboxylase which decarboxylates *p*-coumaric acid into 4-vinylphenol, and the vinylphenol reductase which reduces the latter into 4-ethylphenol.

INTRODUCTION

Phenolic compounds are abundant in wine, originating from the grape material, yeast metabolism and wood barrels. They can be divided in non-flavonoids (which include phenolic acids,) and flavonoids. The two major subgroups of phenolic acids are hydroxybenzoic (gallic and vanillic acids) and hydroxycinnamic (caffeic and ferulic acids) acids.

Hydroxycinnamic acids are the precursors of volatile phenols (4-vinylphenol (4VP), 4-vinylguaiacol, 4-ethylphenol (4EP) and 4-ethylguaiacol). Although they are often regarded as negative, imparting off-flavours to red wines described as "animal", "horse sweat" or "medicinal", some winemakers have considered that, at low concentrations, volatile phenols can contribute positively to the complexity of the bouquet of wines (Fugelsang, 1997).

The yeasts from the genera Dekkera/Brettanomyces are considered to be the main organisms responsible for the production of volatile phenols from hydroxycinnamic acids (Heresztyn, 1986; Chatonnet et al., 1995, 1997). There are, however, some works that report the ability of wine lactic acid bacteria (LAB) to produce volatile phenols (Cavin et al (1993), Chatonnet et al (1995)). Recently, Couto et al (2006) have shown that 37% of 35 LAB strains studied were capable of producing volatile phenols from *p*-coumaric acid.

The conversion of *p*-coumaric acid in 4-ethylphenol involves the sequential activity of two enzymes: the first is the cinnamate decarboxylase and the second is the vinylphenol reductase (Heresztyn, 1986) (Figure 1).

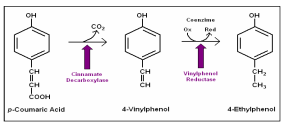


FIGURE 1: Biosynthesis of 4-ethylphenol from *p*-coumaric acid.

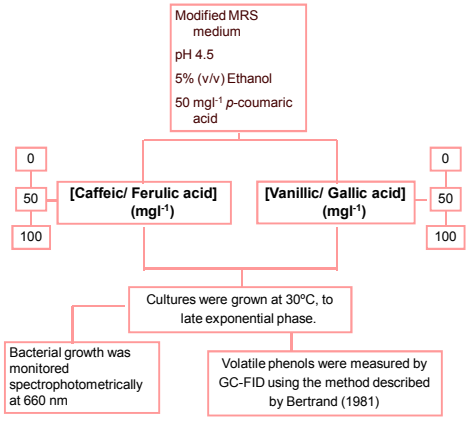
This work aims to study the the effect of hydroxybenzoic and hydroxycinnamic acids on the production of volatile phenols from *p*-coumaric acid by some strains of LAB

MATERIALS AND METHODS

TABLE 1 - List of strains used in this study.

Strains
L. plantarum NCFB 1752
L. collinoides ESB 99
P. pentosaceus NCFB 990
ESB – Escola superior de Biotecnologia Collection, Porto, Portugal NCFB – National Collection of food Bacteria, Reading, England

FIGURE 2 – Schematic representation of the methodology.



RESULTS

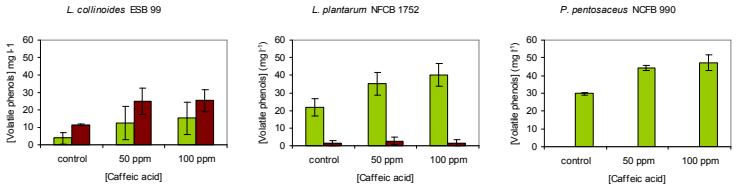


FIGURE 3 – Effect of caffeic acid concentration on the production of 4-vinylphenol (Green bars) and 4-ethylphenol (Bordeaux bars) by *L. collinoides* ESB 99, *L. plantarum* NCFB 1752 and *P. pentosaceus* NCFB 990. Results are the average values of three experiments (with standard deviation).

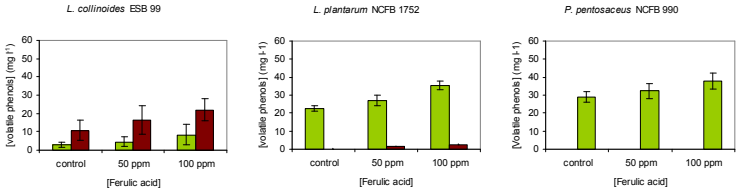


FIGURE 4 – Effect of ferulic acid concentration on the production of 4-vinylphenol (Green bars) and 4-ethylphenol (Bordeaux bars) by *L. collinoides* ESB 99, *L. plantarum* NCFB 1752 and *P. pentosaceus* NCFB 990. Results are the average values of three experiments (with standard deviation).

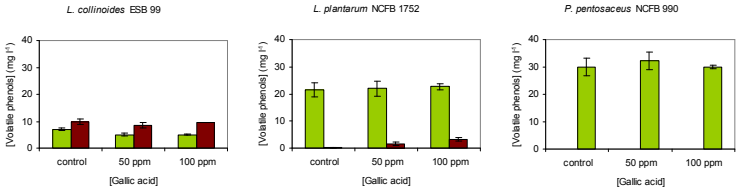


FIGURE 5 – Effect of gallic acid concentration on the production of 4-vinylphenol (Green bars) and 4-ethylphenol (Bordeaux bars) by *L. collinoides* ESB 99, *L. plantarum* NCFB 1752 and *P. pentosaceus* NCFB 990. Results are the average values of three experiments (with standard deviation).

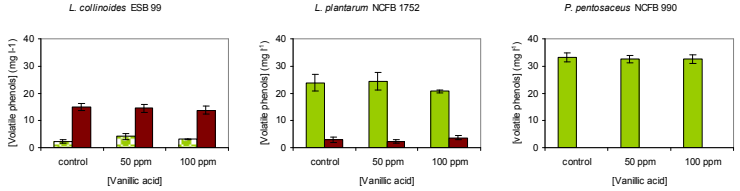


FIGURE 6 – Effect of vanillic acid concentration on the production of 4-vinylphenol (Green bars) and 4-ethylphenol (Bordeaux bars) by *L. collinoides* ESB 99, *L. plantarum* NCFB 1752 and *P. pentosaceus* NCFB 990. Results are the average values of three experiments (with standard deviation).

CONCLUSIONS

- The hydroxycinnamic acids tested (caffeic and ferulic) clearly stimulate the production of volatile phenols from the metabolism of *p*-coumaric acid, while not affecting bacterial growth (Fig. 3 and 4).
- The hydroxybenzoic (gallic and vanillic) acids studied did not significantly affect the growth and the ability of lactic acid bacteria to synthesize volatile phenols (Fig. 5 and 6).
- Previous works have shown the increase on p-coumaric acid decarboxilation when the lactic acid bacteria were grown on a culture medium supplemented with ferulic and *p*-coumaric acids (Beek and Priest, 2000)..
- Apparently hydroxycinnamic acids induce the biosynthesis of enzymes involved in the metabolic pathway: cinnamate decarboxylase which decarboxylates p-coumaric acid into 4-vinylphenol, and the vinylphenol reductase which reduces the latter into 4-ethylphenol.
- Further experiments are required to confirm the possible induction of the enzymes.
- This study highlights the capacity of wine lactic acid bacteria to produce volatile phenols in sensorial significant amounts and that this activity can be affected by certain wine factors.

Acknowledgements:
The authors would like to thank FCT (Fundação para a Ciência e a Tecnologia) for funding this research via project POCI/AGR/61331/2004.

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