THE INFLUENCE OF PH, MALIC ACID AND GLUCOSE ON THE PRODUCTION OF VOLATILE PHENOLS BY WINE LACTIC ACID BACTERIA

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ABSTRACT

In this work, we studied the influence of pH and of the concentration of malic acid and glucose on the ability of lactobacilli and pediococci to produce volatile phenols in culture medium supplemented with *p*-coumaric acid. For most strains, it was noted that the higher the pH the higher the production of volatile phenols in the range of pH values of 3.5-4.5. This behaviour was found to be related with the effect of the pH on the bacterial growth. The influence of malic acid on bacterial activity was found to be strain dependent. In *Lactobacillus plantarum* NCFB1752 and *Lactobacillus collinoides* ESB99, the production of 4-vinylphenol decreased while the production of 4-ethylphenol increased with increasing levels of malic acid in the culture medium. Thus, the 4-vinylphenol reductase activity of these strains seems to be stimulated in the presence of malic acid. The decarboxylase and reductase activity of *Pedicoccus pentosaceus* NCFB990 were not affected by the concentrations of malic acid studied. The concentration of glucose in media strongly influences the production of volatile phenols by *L. collinoides* ESB 99 and *L. plantarum* NCFB 1752, leading to the formation of higher concentrations of 4-ethylphenol and lower concentrations of 4-vinylphenol at low concentrations of glucose (0-5 gl^{-1}) in comparison to 20 gl^{-1}. *P. pentosaceus* NCFB 990 was not affected by the variation of glucose concentration.

INTRODUCTION

➢ Phenolic compounds are abundant in wine, originating from the grape material, yeast metabolism and wood barrels. These compounds include phenolic acids that are the precursors of volatile phenols (4-vinylphenol, 4-vinylguaiacol, 4-ethylphenol 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 conversion of *p*-coumaric acid in 4-ethylphenol involves the sequential activity of two enzymes: the first is the cinnamate decarboxylase which decarboxylates the hydroxycinnamic acid into the 4-vinylphenol (4VP), and the second is the vinylphenol reductase which reduces the latter in 4-ethylphenol (4EP) (Heresztyn, 1986) (Figure 1).

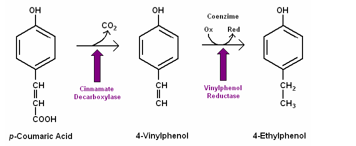


FIGURE 1: Biosynthesis of 4-ethylphenol from *p*-coumaric acid.

➢ The origin of ethylphenols has been under discussion for a long time. 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) tested several strains of LAB isolated from wine for their ability to metabolize *p*-coumaric and ferulic acids, in culture medium. They found that *Lactobacillus* and *Pediococcus* were able to metabolize the phenolic acids with the production of the corresponding ethylphenols. Chatonnet et al (1995), compared the ability of LAB to synthesize volatile phenols with that of *Dekkera/Brettanomyces*. Some of the LAB studied, namely, *P. pentosaceus*, were capable of producing large quantities of 4-vinylphenol but only traces of ethylphenols. Only *L. plantarum* was capable of producing significant quantities of ethylphenols, particularly, 4-ethylphenol, however the concentrations released were very small when compared to those formed by *Dekkera/Brettanomyces* species. Recently, Couto et al (2006) tested the ability of twenty different species of LAB to produce volatile phenols, in culture medium. They have shown that 37% of the strains studied were capable of producing volatile phenols from *p*-coumaric acid.

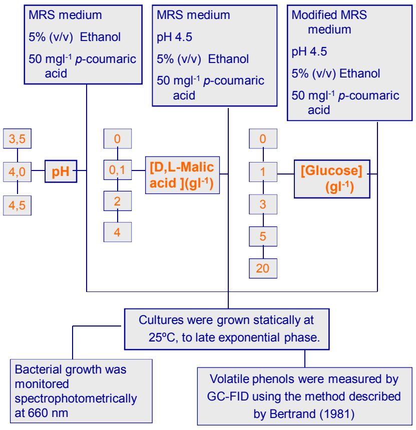
➢ **This work aims to study the influence of certain wine factors on the synthesis of volatile phenols by LAB and on the ratio vinylphenols/ethylphenols produced.**

MATERIALS AND METHODS

TABLE 3 - 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 5 – Schematic representation of the methodology.



RESULTS

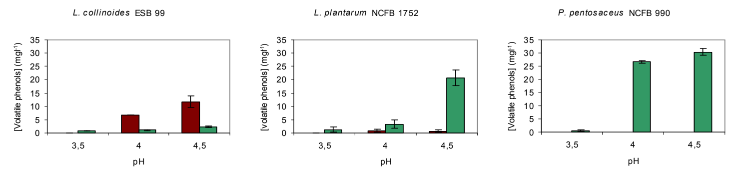


FIGURE 2 – Effect of pH on the production of 4-vinylphenol (Green bars) and 4-ethylphenol (Bordeaux bars) by *L. collinoides* ESB 99, *L. plantarum* NCFB 1752 and *P. pentosaceus* NCFB 990. Results are the average values of two experiments (with standard deviation).

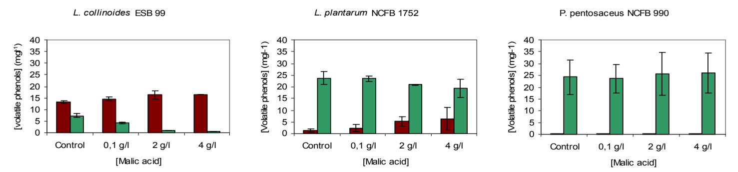


FIGURE 3 – Effect of malic acid concentration on the production of 4-vinylphenol (Green bars) and 4-ethylphenol (Bordeaux bars) by *L. collinoides* ESB 99, *L. plantarum* NCFB 1752 and *P. pentosaceus* NCFB 990. Results are the average values of two experiments (with standard deviation).

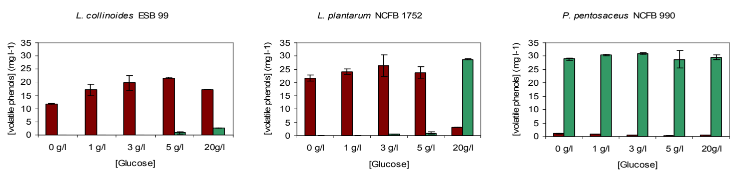


FIGURE 4 – Effect of glucose concentration on the production of 4-vinylphenol (Green bars) and 4-ethylphenol (Bordeaux bars) by *L. collinoides* ESB 99, *L. plantarum* NCFB 1752 and *P. pentosaceus* NCFB 990. Results are the average values of two experiments (with standard deviation).

RESULTS / CONCLUSIONS

▪ In the range of pH values studied, 3.5 to 4.5, it was noted that the higher the pH the higher the production of volatile phenols (Figure 2). This behaviour was found to be related with the effect of pH on bacterial growth but it can be also related with the transport of *p*-coumaric acid or the activity of the enzymes involved in the synthesis of volatile phenols.

▪ Malic acid stimulated the production of 4EP, while diminishing the production of 4VP, by *L. collinoides* ESB 99 and *L. plantarum* NCFB 1752. The conversion of 4VP into 4EP, by the activity of the vinylphenol reductase, may be advantageous in the presence of malic acid, since it presumably produces NAD^+ that is required for the malolactic enzyme activity. The production of volatile phenols by *P. pentosaceus* NCFB 990 was not affected by the different levels of malic acid studied (Figure 3).

▪ The concentration of glucose in media strongly influences the behaviour of *L. collinoides* ESB 99 and *L. plantarum* NCFB 1752. *L. plantarum* NCFB 1752 produces mainly 4VP in the presence of 20 gl^{-1} of glucose, while at 5 gl^{-1} and lower concentrations, 4EP is mainly or solely produced. The capacity of *L. collinoides* ESB 99 to produce 4EP is also higher at 3 and 5 gl^{-1} of glucose than at 20 gl^{-1}.

The results suggest that the vinylphenol reductase activity of these bacteria could be enhanced in the presence of low concentrations of glucose, which may be associated with the cells necessity, in these conditions, to regenerate NAD+, presumably the co-enzyme formed from the reduction of 4VP. As also observed for malic acid, the production of volatile phenols by *P. pentosaceus* NCFB 990 was not affected by the variation of glucose (Figure 4). Low levels of glucose (0-5 gl-1) have strongly inhibited the growth of all strains (results not shown), however they were still able to produce significant amounts of volatile phenols. Further research is needed concerning the effect of glucose on volatile phenol production in these organisms.

▪This study highlights the capacity of wine lactic acid bacteria to produce volatile phenols in sensorially significant amounts and that this activity can be affected by certain wine factors such as pH and the levels of malic acid and glucose.

▪ Further research is required to elucidate the real impact of the metabolic activity of lactic acid bacteria on the levels of volatile phenols found in wines.

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