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

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INTRODUCTION

Phenolic compounds are abundant in wine and they may come from the grape material, may be produced by the yeast metabolism or be extracted from the wood barrels. These compounds include phenolic acids that are the precursors of volatile phenols. Although these compounds, mainly 4-ethylphenol (4EP) and 4-vinylphenol (4VP), 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, they 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, and the second is the vinylphenol reductase which reduces the latter in 4-ethylphenol (Heresztyn, 1986).

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 to produce volatile phenols (Couto et al., 2006), yet they may produce significative amounts of vinylphenols but produce only traces of ethylphenols (Chatonnet et al., 1995, 1997).

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.

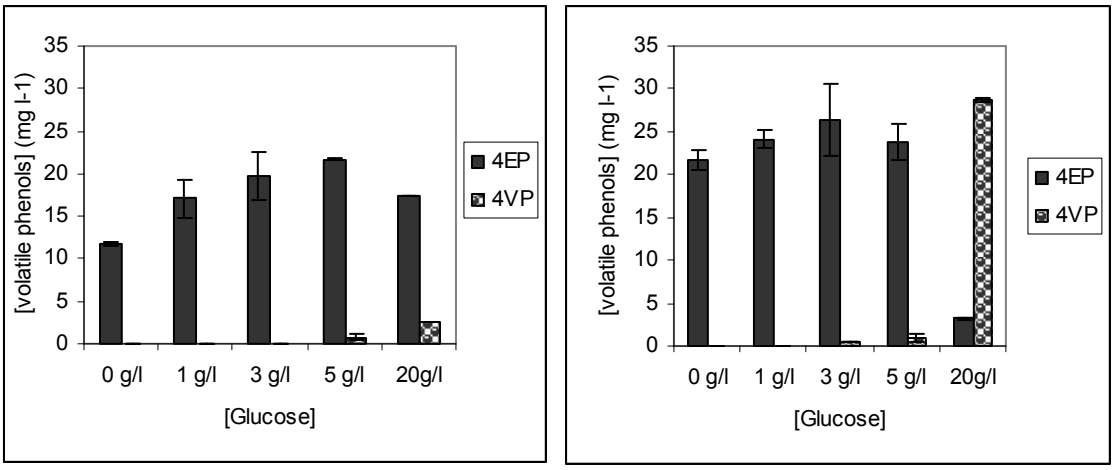
MATERIALS AND METHODS

In this work we used three strains of lactic acid bacteria, *Lactobacillus plantarum* NCFB 1752*, Pediococcus pentosaceus* NCFB 990 (National Culture of Food Bacteria, Reading, England) and *Lactobacillus collinoides* ESB 99 (Escola Superior de Biotecnologia - UCP, Porto, Portugal). These strains were chosen due to their ability to convert *p*-coumaric acid into 4-vinylphenol and/or 4-ethylphenol as demonstrated by Couto et al. (2006). The bacterial growth was monitored spectrophotometrically at 660 nm and the volatile phenols were measured by GC-FID using the method described by Bertrand (1981).

The effect of pH on the activity of the bacteria was studied in MRS medium, with pH values ranging from 3.5 to 4.5. MRS and modified MRS media, at pH 4.5, with final concentrations of malic acid and glucose adjusted to 0, 0.1, 2 and 4 gl^{-1} and 0, 1, 3, 5 and 20 gl^{-1}, respectively, were used to study the influence of these carbohydrates on the production of volatile phenols. In all the experiments the medium was supplemented with 50 mgl^{-1} of *p*-coumaric acid and the final ethanol concentration was adjusted to 5% (v/v). Cultures were grown statically at 25ºC, to late exponential phase. Assays were made in duplicate or triplicate.

RESULTS

(a) (b)



(c)

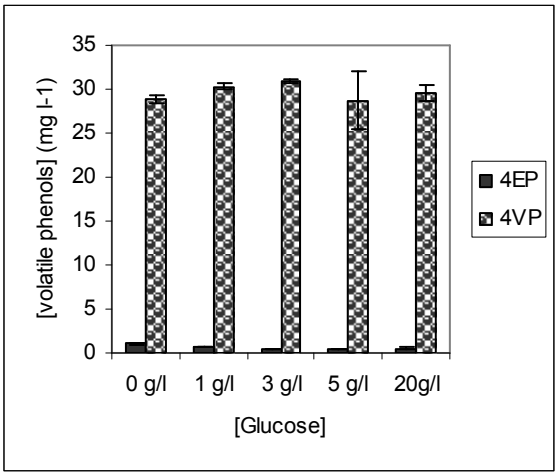


Fig.1 - Effect of glucose concentration on the production of 4-vinylphenol and 4-ethylphenol by (a) *L. collinoides* ESB 99, (b) *L. plantarum* NCFB 1752 and (c) *P. pentosaceus* NCFB 990.

Table 1 – Influence of pH on the production of 4-vinylphenol (4VP) and 4-ethylphenol (4EP) by *L. collinoides* ESB99, *L. plantarum* NCFB 1752 and *P. pentosaceus* NCFB 990 (nd – no detected).

		L. collinoides ESB 99			*L. plantarum* NCFB 1752			*P. pentosaceus* NCFB 990		
		[4VP] (mg l^{-1})	[4EP] (mg l^{-1})	Final OD	[4VP] (mg l^{-1})	[4EP] (mg l^{-1})	Final OD	[4VP] (mg l^{-1})	[4EP] (mg l^{-1})	Final OD
pH	3,5	0,91± 0,01	nd	0,17± 0,01	1,21± 1,04	0,04± 0,00	0,48± 0,54	0,56± 0,39	0,04± 0,00	0,07± 0,01
	4,0	1,08± 0,08	6,71± 0,05	3,35± 0,04	3,27± 1,61	0,90± 0,50	1,27± 0,09	26,69± 0,37	0,05± 0,00	0,87± 0,27
	4,5	2,35± 0,21	11,80± 2,12	4,36± 0,06	20,68± 2,94	0,62± 0,44	2,89± 0,25	30,32± 1,31	0,04± 0,00	1,99± 0,05

Table 2 – Influence of malic acid on the production of 4-vinylphenol (4VP) and 4-ethylphenol (4EP) by *L. collinoides* ESB 99, *L. plantarum* NCFB 1752 and *P. pentosaceus* NCFB 990.

		L. collinoides ESB 99			*L. plantarum* NCFB 1752			*P. pentosaceus* NCFB 990		
		[4VP] (mg l^{-1})	[4EP] (mg l^{-1})	Final OD	[4VP] (mg l^{-1})	[4EP] (mg l^{-1})	Final OD	[4VP] (mg l^{-1})	[4EP] (mg l^{-1})	Final OD
[Malic acid] (g l^{-1})	0	7,34± 0,87	13,18± 0,67	4,40± 0,09	23,76± 2,65	1,37± 0,56	3,73± 0,40	24,32± 7,26	0,21± 0,00	2,59± 0,21
	0.1	4,20± 0,34	14,62± 0,80	4,42± 0,08	23,48± 1,17	2,46± 1,35	3,79± 0,26	23,64± 6,07	0,21± 0,00	2,78± 0,03
	2	1,06± 0,07	16,47± 1,79	4,823± 0,05	20,92± 0,22	5,20± 2,02	4,34± 0,01	25,73± 9,17	0,21± 0,00	3,01± 0,05
	4	0,55± 0,00	16,55± 0,11	5,15± 0,36	19,23± 3,96	6,26± 4,75	4,44± 0,38	25,92± 8,39	0,24± 0,05	3,03± 0,22

DISCUSSION

As it can be seen in Figure 1, the concentration of glucose in media strongly influences the behaviour of *L. collinoides* ESB 99 and *L. plantarum* NCFB 1752 in what concerns the production of volatile phenols. *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 is 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. *P. pentosaceus* NCFB 990 was not affected by the variation of glucose. Low levels of glucose (0 – 5 gl^{-1}) have strongly inhibited the growth of all the 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 this class of organism.

In the range of pH values satudied,3.5 to 4.5, it was noted that the higher the pH the higher the production of volatile phenols (Table 1). 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 (Henick-Kling, T. 1992).

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 produces NAD^+ that is required for the malolactic enzyme activity. As also observed for glucose, the production of volatile phenols by *P. pentosaceus* NCFB 990 was not affected by the different levels of malic acid studied.

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.

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