Influence of Phenolic Acids on Growth and Inactivation of Wine Lactic Acid Bacteria

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ABSTRACT

The effect of several phenolic acids that naturally occur in wines on the growth and viability of *Oenococcus oeni* VF and *Lactobacillus hilgardii* 5 was studied. Five hydroxybenzoic acids and three hydroxycinnamic acids were compared. Hydroxycinnamic acids proved to be more inhibitory to the growth of *O.oeni* than hydroxybenzoic acids. *p*-Coumaric acid had the strongest inhibitory effect on growth of both tested bacteria. On the other hand, some acids (protocatechuic, caffeic and ferulic) showed a beneficial effect on growth of *Lact. hilgardii*, increasing final cell density and decreasing growth rate. Inactivation studies confirmed the apparent inhibitory effect of *p*-coumaric acid on both bacteria. Generally, *O.oeni* was more sensitive to phenolic acid inactivation than *Lact. hilgardii*.

INTRODUCTION

Lactic acid bacteria can be both beneficial and prejudicial for wine quality. These bacteria are responsible for the occurrence of malolactic fermentation in wine, which reduces acidity and improves the sensory characteristics and microbiological stability of wines. *Oenococcus oeni* is the main lactic bacteria responsible for the occurence of malolactic fermentation. Lactic acid bacteria can also act as spoilage microorganisms in wine, namely metabolizing the residual sugars and producing acetic acid, thus reducing wine quality. The ethanol-tolerant bacteria *Lact. hilgardii* has been identified as a major spoilage microorganism in Port wine and other fortified wines (Couto & Hogg, 1994).

The phenolic composition of wines is very diversified and includes phenolic (hydroxycinnamic and hydroxybenzoic) acids in concentrations ranging from 100 to 200 mg l^{-1}, depending on the grape variety and vinification process (Reguant *et al.*, 2000).

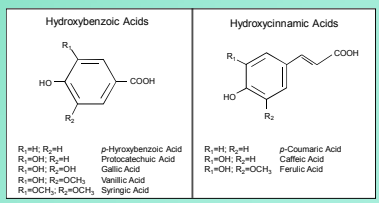

Figure 1 - Structural formulae of some phenolic acids that occur naturally in wines

Despite the structural similarity between acids from hydroxycinnamic and hydroxybenzoic groups, their effect on growth and survival of lactic acid bacteria can be very different. Several studies showed that some free hydroxybenzoic acids can stimulate cell growth and reduce the malolactic fermentation rate and others are slightly inhibitory (Vivas *et al.*, 1997). Other authors report that some free hydroxycinnamic acids inhibit growth of a variety of microorganisms including wine-spoilage strains of *Lact. collinoides* and *Lact. brevis* (Stead, 1993).

In this work, we investigate the effect of five hydroxybenzoic acids and three hydroxycinnamic acids with different aromatic ring substituents on growth and inactivation of *Oenococcus oeni* and *Lactobacillus hilgardii*.

RESULTS

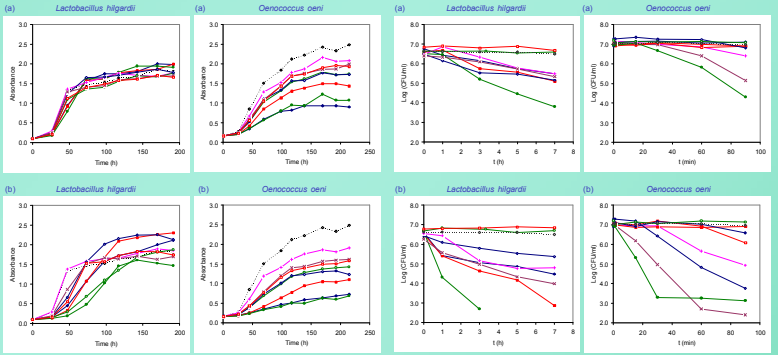

Figure 2 - Growth curves of *Lact. hilgardii 5* and *O. oeni* VF in MRS/TJ media with pH 4.5 and 5% ethanol at 25ºC supplemented with phenolic acids at (a) 200 mg l-1 and (b) 500 mg l-1; each point represents the average value of 3 determinations (RSD<=3%). -◇- Control; -■- *p*-Coumaric acid; -▲- Ferulic acid; -▼- Caffeic acid; -◆- Gallic acid; --○-- *p*-Hydroxybenzoic acid; --□-- Protocatechuic acid; --△-- Vanillic acid; --▽-- Syringic acid

Figure 3 - Inactivation curves of *Lact. hilgardii 5* and *O. oeni* VF in phosphate buffer with pH 4.5 and 9% ethanol at 25ºC supplemented with phenolic acids at (a) 200 mg l-1 and (b) 500 mg l-1; each point represents the average value of 2 determinations. -◇- Control; -■- *p*-Coumaric acid; -▲- Ferulic acid; -▼- Caffeic acid; -◆- Gallic acid; --○-- *p*-Hydroxybenzoic acid; --□-- Protocatechuic acid; --△-- Vanillic acid; --▽-- Syringic acid

MATERIAL AND METHODS

Bacteria and growth conditions

In this experiment we used *Lact. hilgardii* strain ESBUCP 5, isolated from Port wine and *O. oeni* commercial strain VINIFLORA OENOS from CHRISTIAN HANSEN (Hrevidre, Denmark). The liquid growth media (MRS/TJ) used in this experiment was a mixture of MRS (de Man, Rogosa and Sharpe) and TJ (Tomato Juice broth) with pH adjusted to 4.5.

Influence of phenolic acids on growth of *O. oeni* and *Lact. hilgardii*

Cultures were grown to late exponential growth phase in MRS/TJ with 5% (v/v) ethanol at 25ºC, and then transferred to liquid MRS/TJ with 5% ethanol (v/v) containing phenolic acids at 0, 100, 200 and 500 mg l^{-1}. Each individual assay was made in triplicate and incubated at 25ºC. Bacterial growth measurement was determined indirectly by measuring absorbance at 660 nm.

Influence of phenolic acids on inactivation of *O. oeni* and *Lact. hilgardii*

Cultures in stationary phase were centrifuged and the biomass was washed with phosphate buffer (KH_2PO_4, 0.15 M, pH 4.5) with 5% (v/v) ethanol, resuspended in the same phosphate buffer and transfered to Erlenmeyer flasks immersed on a thermostated bath at 25ºC with a magnetic stirrer. Each flask contained phosphate buffer with 9% (v/v) ethanol and phenolic acid at 0, 100, 200 or 500 mg l^{-1}. Samples were collected, properly diluted and plated in duplicate in MRS/TJ media with 20.0 g l^{-1} agar and 5% ethanol. Plates were incubated at 25ºC for 3-5 days.

DISCUSSION

- Phenolic acids can be prejudicial or beneficial to growth of *O.oeni* VF and *Lact. hilgardii* 5, depending on the bacterial species, the phenolic acid and its concentration.
- The inhibitory effect of phenolic acids to growth of *O.oeni* was stronger with hydroxycinnamic acids than with hydroxybenzoic acids. Caffeic and p-coumaric were the most inhibitory acids and gallic acid the least (Fig.2).
- At low concentrations (up to 200 mg l^{-1}) hydroxycinnamic acids led to higher final cell concentrations of *Lact. hilgardii* in spite of a decrease in growth rate and a increase of the initial *lag* phase. This effect was clearly observable for protocatechuic, caffeic and ferulic acids at 500 mg l^{-1}. *p*-Coumaric and *p*-hydroxybenzoic acids caused a decrease of growth rate, but not an increase in cell concentration (Fig. 2).
- Inactivation experiments confirmed the apparent toxicity of *p*-coumaric acid to both bacteria. Syringic, gallic and protocatechuic acid also had an inactivation effect on these bacteria (Fig.3). The higher resistance of *Lact. hilgardii* to inactivation by phenolic acids at 9% ethanol could be a consequence of its higher ethanol tolerance (comparatively to *O.oeni*).
- At 100 mg l^{-1} there were no noticeable effects on growth or inactivation of *Lact. hilgardii*, for all phenolic acids tested (results not shown). At this level of concentration, all phenolic acids showed a slight inhibitory effect on growth of *O.oeni* (results not shown).
- Future work will be focused on the specific mechanisms responsible for growth inactivation and stimulation of wine lactic acid bacteria by phenolic compounds.

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ACKNOWLEDGMENTS

F. Campos would like to thank FCT for the Ph. D. grant PRAXIS XXI BD19909/99.