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Influence of phenolic acids on growth and inactivation of *Oenococcus oeni* and *Lactobacillus hilgardii*

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Keywords: inactivation, lactic acid bacteria, *Lactobacillus hilgardii*, *Oenococcus oeni*, phenolic acids, wine.

ABSTRACT

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Aims: To determine the effect of several wine-associated, phenolic acids on the growth and viability of strains of *Oenococcus oeni* and *Lactobacillus hilgardii*.

Methods and Results: Growth was monitored in ethanol-containing medium supplemented with varying concentrations of hydroxybenzoic acids (*p*-hydroxybenzoic, protocatechuic, gallic, vanillic and syringic acids) and hydroxycinnamic acids (*p*-coumaric, caffeic and ferulic acids). Progressive inactivation was monitored in ethanol-containing phosphate buffer supplemented in a similar manner to the growth experiments. Hydroxycinnamic acids proved to be more inhibitory to the growth of *O. oeni* than hydroxybenzoic acids. On the other hand, some acids showed a beneficial effect on growth of *Lact. hilgardii*. *p*-Coumaric acid showed the strongest inhibitory effect on growth and survival of both bacteria.

Conclusions: Most phenolic acids had a negative effect on growth of *O. oeni*, for *Lact. hilgardii* this effect was only noted for *p*-coumaric acid. Generally, *O. oeni* was more sensitive to phenolic acid inactivation than *Lact. hilgardii*.

Significance and Impact of the Study: Eight wine-derived, phenolic acids were compared for their effects on wine lactic acid bacteria. Results indicate that phenolic acids have the capacity to influence growth and survival parameters. The differences found between phenolic compounds could be related to their different chemical structures.

INTRODUCTION

Lactic acid bacteria are a group of Gram-positive, fermentative, aerotolerant bacteria which produce lactic acid as a major end product from carbohydrate metabolism (Stanier *et al.* 1986). The taxonomy of this group is being reviewed in the light of modern molecular biology (Litopoulos-Tzanetaki and Tzanetakis 2000).

While not being taxonomically discrete, lactic acid bacteria which can interact with the wine environment are often considered together. These bacteria have the ability to tolerate the stresses of the wine environment, namely low pH, presence of ethanol and sulphur dioxide, low temperature and dilute concentration of nutrients (Fleet 1997).

Lactic acid bacteria can interact with a wine, altering the composition in ways which can, under certain circumstances, be considered beneficial to the quality of the final product. This type of 'positive' interaction includes the malolactic fermentation in which, among other reactions, bacteria decarboxylate L-malic acid to L-lactic acid with subsequent alteration in measured and percieved acidity. Some strains of wine lactic acid bacteria can also cause compositional changes which translate into a range of spoilage conditions, from unacceptable acetic acid and ethyl acetate levels to the 'geranium off-flavour' and 'mousy' taint (Sponholz 1992).

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Oenococcus oeni is the main lactic bacteria responsible for the occurrence of desirable forms of the malolactic fermentation (Cavin *et al.* 1993), and strains of this species are the major source of malolactic starter cultures used in the wine industry.

Of the lactic acid bacteria implicated in 'spoilage' conditions, strains of *Lactobacillus* are probably the most cited. In the case of sweet fortified wine, *Lact. hilgardii* has been identified as a major cause of spoilage (Dicks and van Vuuren 1988; Couto and Hogg 1994; de Revel *et al.* 1994).

Phenolic compounds are abundant in wine being extracted from the initial grape material (skins, seeds and stalks) and from wood used for storage. Compounds of this family contribute to the sensory characteristics and chemical qualities of wine both directly and indirectly, through their interactions with other molecule types, e.g. proteins, polysaccharides and other polyphenols (Macheix *et al.* 1990).

The phenolic composition of wines is very complex 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). In wine, phenolic acids appear mainly in a combined form: hydroxycinnamic acids form esters with tartaric acid (cynammoyl–tartaric acids) and hydroxybenzoic acids polymerize with other molecules to produce tannins (Macheix *et al.* 1990). During alcoholic fermentation, free hydroxycynammic acids are released by hydrolysis of tartaric acid esters, but their proportion is relatively low. Despite the structural similarity between acids from the hydroxycinnamic and hydroxybenzoic families, their effect on growth and survival of lactic acid bacteria can be very different. A previous study showed that some free hydroxybenzoic acids can activate cell growth and reduce the malolactic fermentation rate, whereas 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), *Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus* (Herald and Davidson 1983) and several yeasts (Baranowski *et al.* 1980; Stead 1993).

Another study showed that alkyl esters of hydroxycinnamic acids can also have an inhibitory effect on *Pseudomonas fluorescens* (Baranowski and Nagel 1982).

Little work has been published to date concerning the influence of chemical properties of wine phenolic acids and their effect on growth and activity of lactic acid bacteria.

In this work, we investigated the effect of five hydroxybenzoic acids (*p*-hydroxybenzoic, protocatechuic, gallic, vanillic and syringic acids) and three hydroxycinnamic acids (*p*-coumaric, caffeic and ferulic acids) on the growth and inactivation of *O. oeni* and *Lact. hilgardii*.

These compounds exist naturally in wine and were chosen for their different phenolic ring substituents in an attempt to relate the influence of aromatic ring substitution on growth and inactivation of these bacteria.

MATERIALS AND METHODS

Bacteria and growth conditions

Lact. hilgardii strain 5, isolated from Port wine by Couto and Hogg (1994), from the ESBUCP (Escola Superior de Biotecnologia da Universidade Católica Portuguesa, Porto, Portugal) culture collection and *O. oeni* commercial strain Viniflora Oenos from Christian Hansen (Hrevidre, Denmark) were used. *Lact. hilgardii* strain 5 was chosen for being taxonomically representative of the predominant ethanol-tolerant species found in Port wine (Couto and Hogg 1994; Couto 1996).

The liquid growth medium used in this experiment (MRS/TJ) is a mixture (50 : 50) of two commercial media: MRS (de Man, Rogosa and Sharpe) from Biokar Diagnostics (Beauvais, France) and TJ (tomato juice broth) from Difco (Detroit, USA). The initial pH was adjusted to 4·5 with a concentrated (6 M) hydrochloric acid solution before sterilizing. After sterilization (121°C, 15 min), ethanol (99·5% v/v) was added to the medium to obtain a final concentration of 5% v/v ethanol, because this concentration level was found to stimulate growth of both bacteria (Couto 1996).

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

Cultures were grown aerobically, without agitation, to late exponential phase in MRS/TJ with 5% v/v ethanol at 25°C, and then transferred to liquid MRS/TJ containing phenolic acid at 0, 100, 200 and 500 mg l^{-1}. *p*-Hydroxybenzoic, protocatechuic (dihydroxybenzoic), gallic (trihydroxybenzoic), vanillic (*p*-hydroxymethoxybenzoic), syringic (*p*-hydroxydimethoxybenzoic), *p*-coumaric (*p*-hydroxycinnamic), caffeic (dihydroxycinnamic) and ferulic (*p*-hydroxymethoxycinnamic) acids were obtained from Sigma-Aldrich (Steinheim, Germany). Fresh concentrated solutions (10 g l^{-1}) of phenolic acids were prepared in pure (99·5% v/v) ethanol and added to the growth media. The final ethanol concentration of the media was adjusted to 5% v/v.

Each individual assay was made in triplicate and incubated aerobically and without agitation at 25°C. All assays were made simultaneously and the whole experiment was repeated to verify the results.

Growth measurement

Bacterial growth measurement was determined indirectly by measuring absorbance at 660 nm, using an UV/VIS Unicam 8620 spectrophotometer (Unicam, Cambridge, UK) and

optical cells of 1 cm path length. Dilutions with distilled water were made when the optical density value exceeded the linearity limit of Beer–Lambert's law.

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

The effect of different phenolic acids on the inactivation of these lactic bacteria was tested in phosphate buffer solution (at pH 4·5) with 9% v/v ethanol. This concentration level was chosen because it represents an ethanol stress while generating inactivation kinetics which are measurable over a convenient time period. Cultures in stationary phase (grown aerobically, without agitation, for four days in MRS/TJ with 5% (v/v) ethanol at 25°C) were centrifuged (10 min, 3000 *g*). The biomass was washed with phosphate buffer (KH_2PO_4, 0·15 M, pH 4·5) containing 5% (v/v) ethanol and centrifuged again.

Cells were resuspended in phosphate buffer containing 5% v/v ethanol and transfered to 250 ml Erlenmeyer flasks, stirred magnetically and immersed in a thermostatted water bath at 25°C. Each flask contained 100 ml of phosphate buffer containing 9% (v/v) ethanol and phenolic acid at 0, 100, 200 or 500 mg l^{-1}.

Samples were collected at 1, 15, 30, 60 and 90 min (in the case of *O. oeni*) and at 0, 1, 3, 5 and 7 h (in the case of *Lact. hilgardii*) after inoculation of the phosphate buffer, properly diluted and plated in duplicate on MRS/TJ media containing 20·0 g l^{-1} Agar MC2 (Lab M, Bury, UK) and 5% ethanol. Plates were incubated aerobically at 25°C for 5–7 days.

The whole experiment was repeated for each phenolic acid and bacterium to confirm the results.

RESULTS

Effect of phenolic acids on growth of *O. oeni*

All phenolic acids tested in this experiment were inhibitory for the growth of O. *oeni*, although the inhibitory effect was stronger with hydroxycinnamic acids than with hydroxybenzoic acids. This inhibitory effect increased with the concentration of phenolic acids and caused a decrease in both the maximal growth rate and maximal cell density when compared to the control culture (Fig. 1).

In the case of the hydroxycinnamic acids, the inhibitory effect was greatest for caffeic and *p*-coumaric acids and least for ferulic acid (Fig. 1a,b,c).

In the case of the hydroxybenzoic acids, the inhibitory effect was greatest with protocatechuic and *p*-hydroxybenzoic acids and least with gallic acid. Vanillic and syringic acids showed an intermediate effect (Fig. 1d,e,f).

Effect of phenolic acids on growth of *Lact. hilgardii*

All phenolic acids tested in this experiment did not affect significantly the growth of *Lact. hilgardii* when present at 100 mg l^{-1} (Fig. 2a,d).

In the case of hydroxycinnamic acids, at 200 mg l^{-1} there was a decrease in the growth rate and a slight increase in the final cell concentration of the cultures, when compared to the control (Fig. 2b). At 500 mg l^{-1} there was an increase in the lag phase and a decrease in the growth rate of the cultures. This effect was stronger with *p*-coumaric acid than with caffeic and ferulic acids. Cultures supplemented with caffeic and ferulic acids reached higher final cell concentrations than the control culture (Fig. 2c).

In the case of hydroxybenzoic acids at 500 mg l^{-1}, gallic acid showed no effect on growth of *Lact. hilgardii*, while vanillic and syringic acids caused a decrease in growth rate, comparatively with the control culture (Fig. 2f). At this concentration, *p*-hydroxybenzoic acid showed an inhibitory effect similar to *p*-coumaric acid (decrease in growth rate) and protocatechuic acid a similar effect to ferulic acid (decrease in growth rate and increase in cell concentration).

Influence of phenolic acids on inactivation of *Lact. hilgardii*

At 100 mg l^{-1}, only *p*-coumaric and gallic acids showed a slight inhibitory effect on *Lact. hilgardii* (Fig. 3a, Table 1). At higher concentrations, several acids had the same effect on the bacteria. At 500 mg l^{-1}, *p*-coumaric acid caused a sharp decrease of viable bacteria, inactivating most cells after 7 h (Fig. 3c, Table 1). At this concentration level, all the phenolic acids tested showed an inhibitory action, except vanillic and *p*-hydroxybenzoic acids. *p*-Coumaric acid caused the highest decrease in viability at all concentrations tested.

Influence of phenolic acids on inactivation of *O. oeni*

Results obtained indicate that at concentration of 100 mg l^{-1} there was no noticeable inactivation effect on *O. oeni* for any phenolic acid (Fig. 3d, Table 2). At 200 mg l^{-1} *p*-coumaric and syringic acids inactivated *O. oeni*, causing a noticeable decrease of viable cells after 90 min (Fig. 3e, Table 2). At 500 mg l^{-1}, several phenolic acids caused inactivation of the bacteria, *p*-coumaric and syringic acids having the highest effects. Ferulic, caffeic, *p*-hydroxybenzoic and vanillic acids did not have significant inactivation effects on the bacteria, even at 500 mg l^{-1} (Fig. 3f, Table 2).

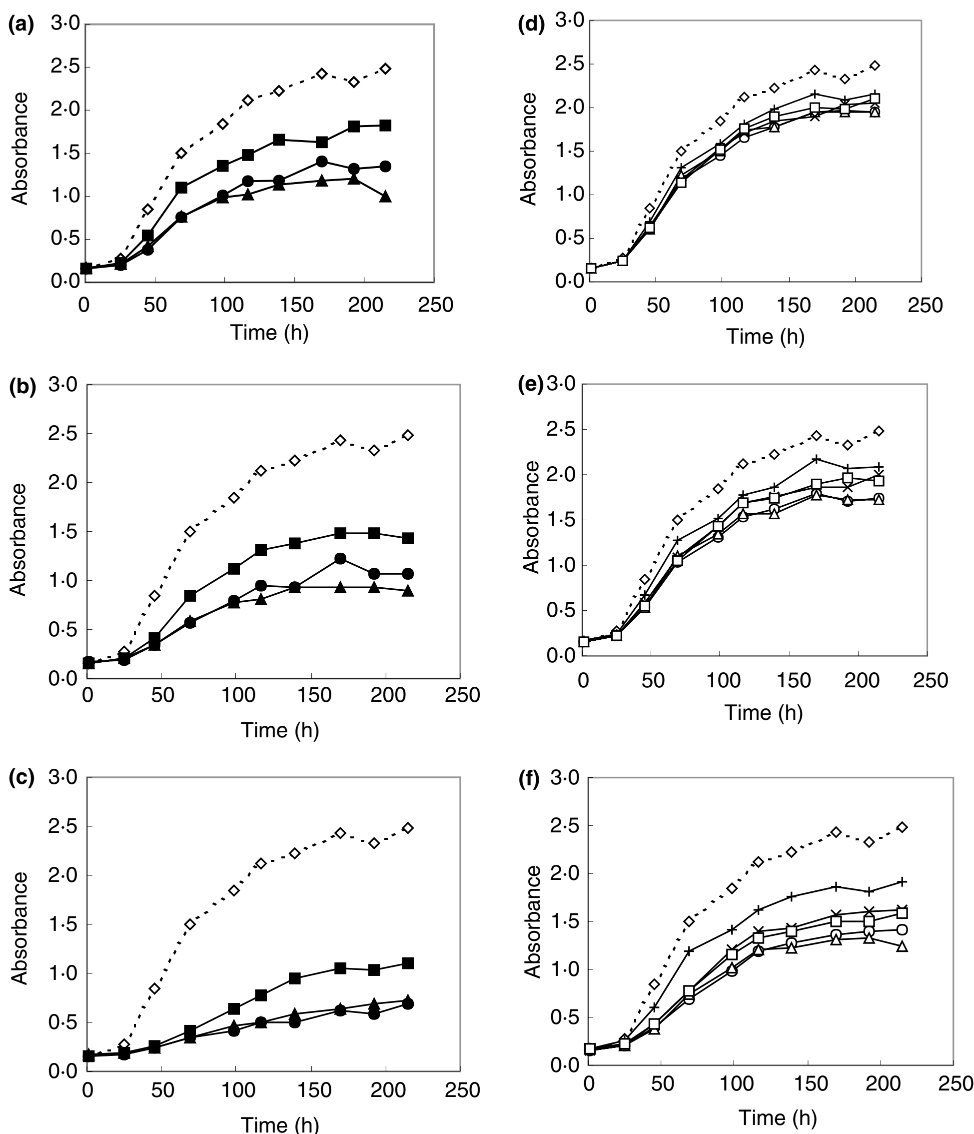

Fig. 1 Growth curves of *Oenococcus oeni* VF in MRS/TJ media (pH 4·5, 5% v/v ethanol at 25°C) supplemented with hydroxycinnamic acids at: (a) 100 mg l^{-1} (b) 200 mg l^{-1} (c) 500 mg l^{-1} and hydroxybenzoic acids at (d) 100 mg l^{-1} (e) 200 mg l^{-1} (f) 500 mg l^{-1}. (◇) Control, (●) *p*-coumaric acid, (▲) caffeic acid, (■) ferulic acid, (○) *p*-hydroxybenzoic acid, (Δ) protocatechuic acid, (+) gallic acid (□) vanillic acid, (×) syringic acid; each point represents the average value of three determinations (RSD < = 3%)

DISCUSSION

The results indicate that phenolic acids influence the growth of *O. oeni* VF and *Lact. hilgardii* 5, in growth media. This influence can be either positive or negative in terms of growth stimulation, depending on the bacterial species, the specific phenolic acid used and its concentration.

All phenolic acids tested were inhibitory for the growth of O. *oeni* VF, even at the lowest concentration tested (100 mg l^{-1}), although the inhibitory effect was stronger with hydroxycinnamic acids than with hydroxybenzoic acids. Caffeic and *p*-coumaric were the most inhibitory compounds, having effects of similar magnitude on the growth of this bacterium. On the other hand, gallic acid had the least inhibitory effect of all phenolic acids tested.

Results obtained with *Lact. hilgardii* 5 show that, at low concentrations (up to 200 mg l^{-1}) hydroxycinnamic acids led to higher final cell concentrations in spite of a decrease in growth rate and a lengthening of the initial lag phase. These effects were also observable at 500 mg l^{-1}, except for *p*-coumaric acid, which had a strong negative effect on

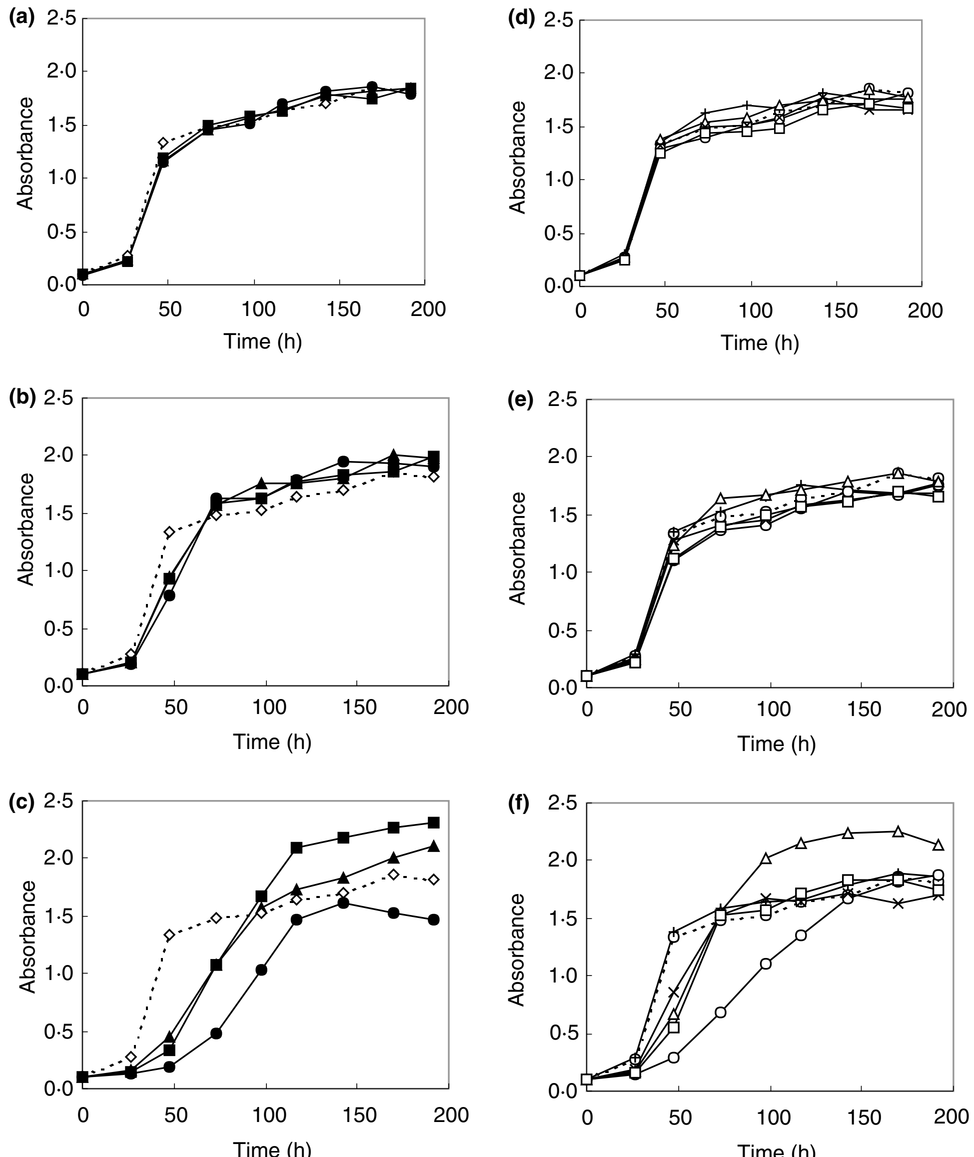

Fig. 2 Growth curves of *Lactobacillus hilgardii* 5 in MRS/TJ media (pH 4·5, 5% v/v ethanol at 25°C) supplemented with hydroxycinnamic acids at: (a) 100 mg l^{-1}, (b) 200 mg l^{-1}, (c) 500 mg l^{-1} and hydroxybenzoic acids at (d) 100 mg l^{-1}, (e) 200 mg l^{-1}, (f) 500 mg l^{-1}. (◇) Control, (●) *p*-coumaric acid, (▲) caffeic acid, (■) ferulic acid, (○) *p*-hydroxybenzoic acid, (Δ) protocatechuic acid, (+) gallic acid, (□) vanillic acid, (×) syringic acid; each point represents the average value of three determinations (RSD < = 3%)

bacterial growth. These results agree, in essence, with those reported previously in experiments conducted with other lactobacilli (Stead 1993).

Phenolic acids have different antioxidant (oxygen radical scavenger) capacities which are related to their aromatic ring substituents (Natella *et al.* 1999). This antioxidant effect is stronger with cinnamic than with benzoic acids and is highest with dihydroxy (caffeic and protocatechuic) and *p*-hydroxymethoxy (ferulic and vanillic) substituents. This antioxidant capacity of these compounds might be involved in some way in the stimulation effect of these compounds on growth of *Lact. hilgardii* 5.

Some lactobacilli can metabolize hydroxycinnamic acids, originating 2-hydroxyphenylpropionic acids which can, in turn, be decarboxylated yielding substituted *p*-ethyl phenols (Cavin *et al.* 1993; Stead 1993; Chatonnet *et al.* 1995, 1997). These compounds are often described as off-flavours in wine and have very low sensory thresholds (Chatonnet *et al.* 1997). The metabolism of certain hydroxycinnamic acids could also be a possible explanation for the beneficial effect

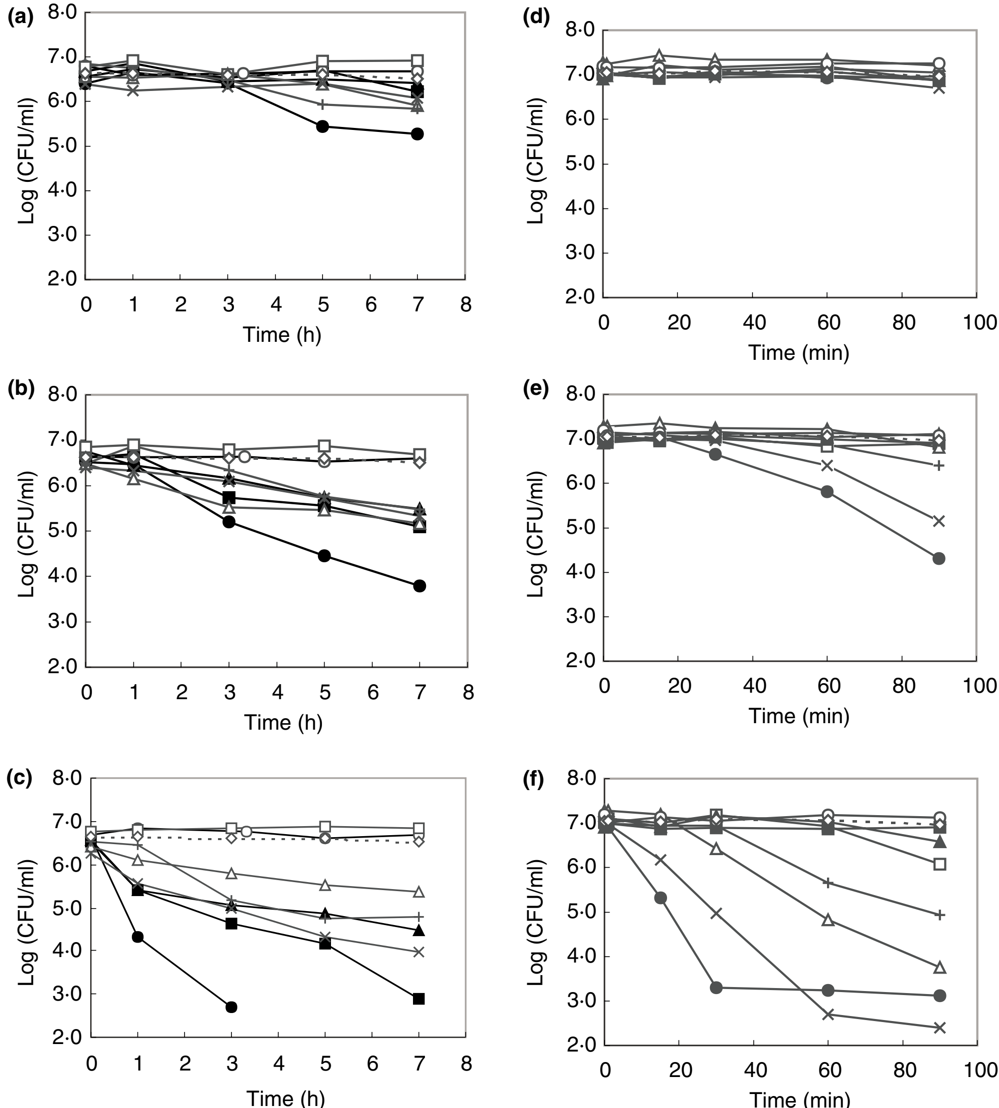

Fig. 3 Inactivation curves of wine lactic acid bacteria in phosphate buffer (pH 4·5, 9% v/v ethanol at 25°C) supplemented with phenolic acids. Inactivation of *Lactobacillus hilgardii* 5 at (a) 100 mg l^{-1} (b) 200 mg l^{-1} (c) 500 mg l^{-1} phenolic acid concentration and *Oenococus oeni* VF at (d) 100 mg l^{-1} (e) 200 mg l^{-1} (f) 500 mg l^{-1} phenolic acid concentration. (◇) Control, (●) *p*-coumaric acid, (▲) caffeic acid, (■) ferulic acid, (○) *p*-hydroxybenzoic acid, (△) protocatechuic acid, (+) gallic acid, (□) vanillic acid, (×) syringic acid

of these compounds on growth of *Lact. hilgardii* 5. Further investigations are necessary to evaluate the potential of this bacterium to produce volatile *p*-ethyl phenols and to understand the biological motives of these conversions.

Hydroxycinammic acids, due to their propenoic side chain, are much less polar than the corresponding hydroxybenzoic acids, and this property might facilitate the transport of these molecules across the cell membrane, which might be related in turn to the stronger inhibitory effect of hydroxycinammic acids in the experiments performed with *O.oeni*. The polarity of the molecule alone, however, cannot explain the different effects of the phenolic acids. In fact, the most inhibitory hydroxycinammic and hydroxybenzoic acids to bacterial growth were *p*-coumaric and *p*-hydroxybenzoic acids, respectively, exhibiting intermediate polarities within each group. Some authors suggest that an equilibrium between solubility in both lipid and aqueous phases could be necessary to ensure the inhibitory effect of hydroxycinammic acids on bacterial growth (Herald and Davidson 1983).

Inactivation experiments confirmed the apparent toxicity of *p*-coumaric acid to both bacteria. Syringic, gallic and

Table 1 Effect of phenolic acid concentration on inactivation of *Lactobacillus hilgardii* 5 in phosphate buffer (pH 4·5, 9% v/v ethanol) at 25°C

	0		100		200		500	
Concentration (mg l^{-1})	log n/log n_0*	SD	log n/log n_0	SD	log n/log n_0	SD	log n/log n_0	SD
p-Hydroxybenzoic acid	0·96	±0·01	0·98	±0·02	1·00	±0·02	1·00	±0·01
Protocatechuic acid	1·00	±0·01	0·93	±0·05	0·80	±0·04	0·76	±0·08
Gallic acid	1·00	±0·01	0·85	±0·09	0·85	±0·02	0·73	±0·05
Vanillic acid	1·01	±0·01	1·02	±0·01	0·97	±0·05	1·01	±0·03
Syringic acid	0·98	±0·01	0·96	±0·01	0·84	±0·03	0·64	±0·01
p-Coumaric acid	0·97	±0·02	0·83	±0·01	0·58	±0·02	< 0·41†	–
Caffeic acid	1·00	±0·01	0·96	±0·02	0·81	±0·02	0·68	±0·00
Ferulic acid	0·95	±0·04	0·94	±0·01	0·78	±0·01	0·44	±0·03

*n_0, initial viable cell number; n, viable cell number after 7 h; SD, standard deviation; values represent the average of four determinations; †value obtained after 3 h.

Table 2 Effect of phenolic acid concentration on inactivation of *Oenococcus oeni* VF in phosphate buffer (pH 4·5, 9% v/v ethanol) at 25°C

	0		100		200		500	
Concentration (mg l^{-1})	log n/log n_0*	SD	log n/log n_0	SD	log n/log n_0	SD	log n/log n_0	SD
p-Hydroxybenzoic acid	1·00	±0·07	1·01	±0·04	1·01	±0·04	0·92	±0·14
Protocatechuic acid	0·99	±0·06	0·94	±0·09	0·96	±0·04	0·47	±0·06
Gallic acid	0·98	±0·03	0·96	±0·06	0·93	±0·04	0·69	±0·02
Vanillic acid	1·01	±0·02	0·99	±0·03	0·97	±0·04	0·89	±0·04
Syringic acid	1·01	±0·02	0·91	±0·05	0·77	±0·05	0·35	±0·01
p-Coumaric acid	1·00	±0·02	0·99	±0·03	0·61	±0·04	0·36	±0·11
Caffeic acid	0·98	±0·04	0·99	±0·02	0·97	±0·05	0·85	±0·10
Ferulic acid	0·99	±0·07	0·98	±0·07	0·99	±0·06	0·93	±0·12

*n_0, initial viable cell number; n, viable cell number after 90 min; SD, standard deviation; values represent the average of four determinations.

protocatechuic acid also had an inactivation effect on these organisms.

Generally, *Lact. hilgardii* 5 was much more resistant than *O. oeni* VF to inactivation by phenolic acids at 9% ethanol (v/v) which could be explained by the fact that the latter is much less ethanol-tolerant than the former. The kinetics of inactivation of *Lact. hilgardii* were determined over a longer period of time than that of *O. oeni* because of its higher tolerance to ethanol.

The inhibitory effect of some phenolic acids apparently cannot be explained by the simple adsorption to cell walls (Vivas *et al.* 1997). Other mechanisms proposed include a role as cytoplasmic poisons, due to their putative ability to enter the cell in undissociated form, dissociating in the cytoplasm and hence being progressively accumulated (Reguant *et al.* 2000). It is known, of course, that generically phenolic compounds can damage the bacterial cell membrane, causing leakage of intracellular constituents (McDonnell and Russell 1999). Hydroxycinammic acid derivatives are known to interact with the membrane lipids of taste papillae in the tongue, causing a bitter sensation (Macheix *et al.* 1990). This bitterness is caused presumably by a neutralization of the membrane electric potential, following penetration of the molecule. A similar effect could occur in bacterial cell membranes, affecting their energy metabolism. Some polyphenols can interact with carbohydrates and proteins by hydrogen bonding, hydrophobic and ionic interactions (McManus *et al.* 1985). Interaction with cell enzymes could also be a possible explanation for the inhibitory effect of phenolic acids.

The above conclusions are largely speculation, which shows that a general lack of knowledge exists concerning this specific subject. That phenolic acids have the capacity to influence growth and survival parameters has been shown. The effects these compounds might exert on other activity parameters, such as metabolism, are also beginning to be described (Vivas *et al.* 1997; Reguant *et al.* 2000). The specific mechanisms which underlie these effects are far from being described fully.

Current work is being focused on the specific mechanisms responsible for inactivation or growth stimulation of wine lactic acid bacteria by phenolic compounds.

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REFERENCES

Baranowski, J.D., Davidson, P.M., Nagel, C.W. and Branen, A.L. (1980) Inhibition of *Saccharomyces cerevisiae* by naturally occurring hydroxycinnamates. *Journal of Food Science* **45**, 592–594.

Baranowski, J.D. and Nagel, C.W. (1982) Inhibition of *Pseudomonas fluorescens* by hydroxycinammic acids and their alkyl esters. *Journal of Food Science* **47**, 1587–1589.

Cavin, J.F., Andioc, V., Etievant, P.X. and Divies, C. (1993) Ability of wine lactic acid bacteria to metabolize phenol carboxylic acids. *American Journal of Enology and Viticulture* **44**, 76–80.

Chatonnet, P., Dubourdieu, D. and Boidron, J.N. (1995) The influence of *Brettanomyces/Dekkera sp.*, yeasts and lactic acid bacteria on the ethylphenol content of red wines. *American Journal of Enology and Viticulture* **46**, 463–468.

Chatonnet, P., Viala, C. and Dubourdieu, D. (1997) Influence of polyphenolic components of red wines on the microbial synthesis of volatile phenols. *American Journal of Enology and Viticulture* **48**, 443–448.

Couto, J.A. (1996) Studies on diversity, taxonomy and physiology of ethanol tolerant *Lactobacilli* isolated from Douro fortified wine *PhD Thesis*, *Escola Superior de Biotecnologia da Universidade Catòlica Portuguesa*, Porto, Portugal.

Couto, J.A. and Hogg, T.A. (1994) Diversity of ethanol-tolerant lactobacilli isolated from Douro fortified wine: clustering and identification by numerical analysis of electrophoretic protein profiles. *Journal of Applied Bacteriology* **76**, 487–491.

Dicks, L.M.T. and van Vuuren, H.J.J. (1988) Identification and physiological characteristics of heterofermentative strains of *Lactobacillus* from South African red wines. *Journal of Applied Bacteriology* **64**, 505–513.

Fleet, G.H. (1997) Wine. In *Food Microbiology Fundamentals and Frontiers* ed. Doyle, M.P., Beuchat, L.R. and Montville, T.J., pp. 682. Washington DC: ASM Press.

Herald, P.J. and Davidson, P.M. (1983) Antibacterial activity of selected hydroxycinammic acids. *Journal of Food Science* **48**, 1378–1379.

Litopoulos-Tzanetaki, E. and Tzanetakis, N. (2000) Fermented milks. In *Encyclopedia of Food Microbiology* ed. Robinson, R.R., Batt, C.A. and Patel, P.D., Vol. 2, pp. 774, 777. London: Academic Press.

Macheix, J.J., Fleuriet, A. and Billot, J. (1990) *Fruit Phenolics*, pp. 323–332. Boca Raton, Florida: CRC Press.

McDonnell, G. and Russell, A.D. (1999) Antiseptics and disinfectants: activity, action, and resistance. *Clinical Microbiology Reviews* **12**, 147–179.

McManus, J.P., Davis, K.G., Beart, J.E., Gaffney, S.H. and Lilley, T.H. (1985) Polyphenol interactions. Part 1. Introduction: some observations on the reversible complexation of polyphenols with proteins and polysaccharides. *Journal of the Chemical Society Perkin Transactions* **II**, 1429–1438.

Natella, F., Nardini, M., Di Felice, M. and Scaccini, C. (1999) Benzoic and cinnamic acid derivatives as antioxidants: structure–activity relation. *Journal of Agriculture and Food Chemistry* **47**, 1453–1459.

Reguant, C., Bordons, A., Arola, L. and Rozès, N. (2000) Influence of phenolic compounds on the physiology of *Oenococcus oeni* from wine. *Journal of Applied Microbiology* **88**, 1065–1071.

de Revel, G., Capela, A.B. and Hogg, T. (1994) A pre-spoilage marker for bacterial activity in fortified wines, conversion of L-malic acid to L-lactic acid. *Letters in Applied Microbiology* **18**, 329–332.

Sponholz, W.R. (1993) Wine spoilage by microorganisms. In *Wine Microbiology and Biotechnology* ed. Fleet G., pp. 404–413. Chur: Harwood Academic Publishers.

Stanier, R.Y., Ingraham, J.L., Wheelis, M.L. and Painter, P.R. (1986) *General Microbiology*, 5th edn, pp. 496–497. London: MacMillan Press.

Stead, D. (1993) The effect of hydroxycinnamic acids on the growth of wine-spoilage lactic acid bacteria. *Journal of Applied Microbiology* **75**, 135–141.

Vivas, N., Lonvaud-Funel, A. and Glories, Y. (1997) Effect of phenolic acids and anthocyanins on growth, viability and malolactic activity of a lactic acid bacterium. *Food Microbiology* **14**, 291–300.