Effect of phenolic aldehydes and flavonoids on growth and inactivation of *Oenococcus oeni* and *Lactobacillus hilgardii*

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Abstract

The aim of this work was to investigate the effect of wine phenolic aldehydes, flavonoids and tannins on growth and viability of strains of *Oenococcus oeni* and *Lactobacillus hilgardii*. Cultures were grown in ethanol-containing MRS/TJ medium supplemented with different concentrations of phenolic aldehydes or flavonoids and monitored spectrophotometrically. The effect of tannins was evaluated by monitoring the progressive inactivation of cells in ethanol-containing phosphate buffer supplemented with grape seed extracts with different molecular weight tannins. Of the phenolic aldehydes tested, sinapaldehyde, coniferaldehyde, *p*-hydroxybenzaldehyde, 3,4-dihydroxybenzaldehyde and 3,4,5-trihydroxybenzaldehyde significantly inhibited the growth of *O. oeni* VF, while vanillin and syringaldehyde had no effect at the concentrations tested. *Lact. hilgardii* 5 was only inhibited by sinapaldehyde and coniferaldehyde. Among the flavonoids, quercetin and kaempferol exerted an inhibitory effect especially on *O. oeni* VF. Myricetin and the flavan-3-ols studied (catechin and epicatechin) did not affect considerably the growth of both strains. Condensed tannins (particularly tetramers and pentamers) were found to strongly affect cell viability, especially in the case of *O. oeni* VF. In general, this strain was found to be more sensitive than *Lact. hilgardii* 5 to the phenolic compounds studied. This work contributes to the knowledge of the effect of different phenolic compounds on the activity of wine lactic acid bacteria, which, especially in the case of aldehydes and of different molecular weight fractions of tannins, is very scarce.

Introduction

Wine is a harsh environment for microorganisms, mainly due to its low pH, low availability of nutrients and the presence of ethanol and sulphur dioxide. However, certain microorganisms, among which some genera of lactic acid bacteria, have the ability to tolerate the wine stresses (Fleet et al., 1997). In wine, these bacteria, which occur in four genera (*Oenococcus*, *Pediococcus*, *Lactobacillus* and *Leuconostoc*), can have a positive effect, being responsible for the malolactic fermentation, but can also have deleterious effects, being responsible for a range of wine spoilage conditions. In the malolactic fermentation the main reaction is the decarboxylation of L-malic acid to L-lactic acid, leading to deacidification, flavour modification and increased microbial stability of wine (Henick-Kling, 1993). *Oenococcus oeni* is the main lactic acid bacteria responsible for the malolactic fermentation, being often chosen as a starter culture in commercial wine applications (Van Vuuren and Dicks, 1993; Coucheney et al., 2005). Wine spoilage can be a consequence of the unrestrained growth of lactic acid bacteria and depending on the wine and bacterial species, different types of faults can take place (Sponholz, 1993). The genus *Lactobacillus* is often involved in the spoilage of wines; particularly, the species *Lactobacillus hilgardii* has been identified as a major cause of spoilage of fortified wines (Couto and Hogg, 1994; de Revel et al., 1994).

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Phenolic compounds have a major contribution to the sensory characteristics of wines, namely colour, mouthfeel, bitterness and astringency. These compounds, which derive from grape material (skins, seeds and stalks) and from the wood used in wine storage (Ribéreau-Gayon et al., 2000), can be divided into two groups: non-flavonoids (which includes phenolic acids, aldehydes and alcohols) and flavonoids (comprising anthocyanins, flavonols and flavanols) (Macheix et al., 1990). Most non-flavonoids have a simple structure (Fig. 1), possessing one or more hydroxy and methoxy groups directly bound to the benzene ring. Phenolic aldehydes are structurally similar to phenolic acids and originate from the degradation of lignins in wood cooperage (Jackson, 2000). Flavonoids possess the flavilium ring C_6–C_3–C_6 as structural backbone (Fig. 1). Among them, the flavon-3-ols are present mainly in the glycoside form and can be differentiated according to the hydroxylation of the B-ring: kaempferol (1 OH), quercetin (2 OH) and myricetin (3 OH) (Macheix et al., 1990; Ribéreau-Gayon et al., 2000). The most important flavan-3-ols in wine are (+)-catechin and (−)-epicatechin, which are the basic units of procyanidins and condensed tannins (Macheix et al., 1990). Unlike flavon-3-ols, flavan-3-ols have a characteristic saturated C-ring. Tannins are defined as polymers with undefined and complex structure capable of producing stable interactions with proteins and other plant polymers such as polysaccharides. Depending on their basic structural unit, tannins can be divided into hydrolysable tannins (with gallic acid, ellagic acid or its derivates as monomers) and condensed or catechic tannins (with catechins and epicatechins as monomers) (Macheix et al., 1990; Ribéreau-Gayon et al., 2000).

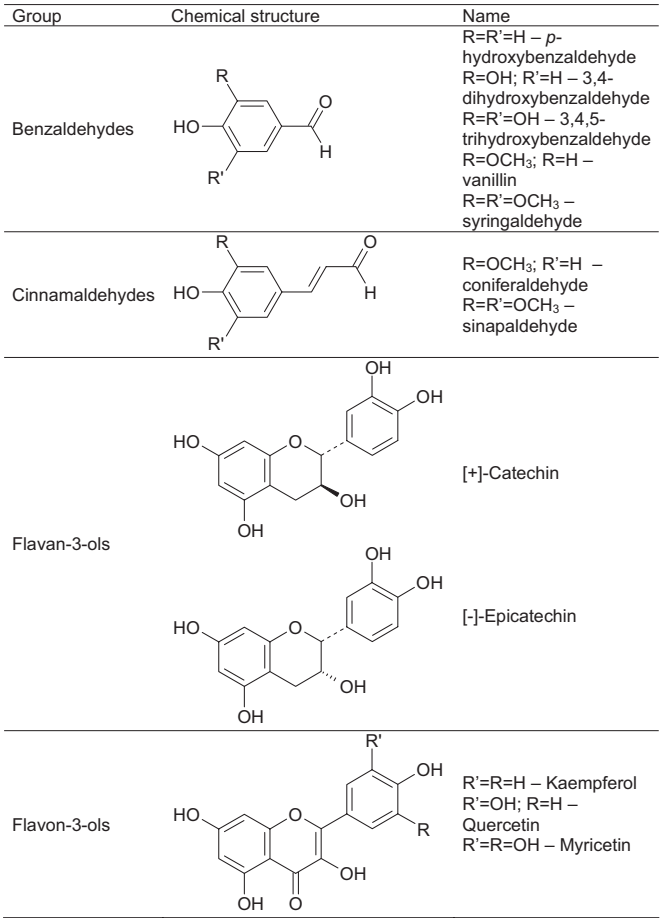

Fig. 1. Chemical structure of phenolic compounds.

Some studies started to unveil the biological effect of phenolic compounds on wine microorganisms. It has been found that phenolic extracts from wood and wines have a stronger influence on the growth of microorganisms than some of their individual components (Vivas et al., 2000; Papadopoulou et al., 2005). The influence of these compounds on lactic acid bacteria remains unclear; while some stimulate bacterial growth and activity, others have an inhibitory effect. It has been described that catechin stimulates the growth and the malolactic fermentation activity of *O. oeni* and *Lact. hilgardii* (Reguant et al., 2000; Alberto et al., 2001). Vivas et al. (2000) found that procyanidins adversely affected the viability of *O. oeni*. Some phenolic acids were found to influence negatively the growth and survival of wine lactic acid bacteria (Stead, 1993; Campos et al., 2003). Phenolic aldehydes have been found to exert a toxic effect on microorganisms (Barber et al., 2000; Puupponen-Pimiä et al., 2001; Friedman et al., 2003; Fitzgerald et al., 2004; Gill and Holley, 2004) but very little is known about its action on wine bacteria.

In the present work, we studied the effect of phenolic aldehydes, flavonols, flavanols and tannins on the growth or cell viability of *O. oeni* VF and *Lact. hilgardii* 5. This is, to our knowledge, the first work employing different molecular weight fractions (obtained from grape seed extractions) to study the influence of tannins on the cell viability of wine lactic acid bacteria.

Materials and methods

Bacteria and growth conditions

Lact. hilgardii strain 5, isolated 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 employed in this study. *Lact. hilgardii* strain 5 was chosen for being taxonomically representative of the predominant ethanol-tolerant species found in Port wine (Couto, 1996; Couto and Hogg, 1994).

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 mol L^{-1}) hydrochloric acid solution. Ethanol (99.5%, v/v) was added to the medium after sterilization (121 °C, 15 min) to obtain a final concentration of 5% (v/v). This concentration of

ethanol in the culture medium was found to stimulate the growth of these organisms (Couto, 1996). Cultures were grown aerobically, without agitation at 25 °C.

Inuence of phenolic aldehydes and flavonoids on the growth of O. oeni and Lact. hilgardii

Cultures were grown to late exponential phase in MRS/TJ with 5% (v/v) ethanol and then transferred to liquid MRS/TJ containing phenolic compounds (aldehydes and flavonoids). All these compounds were obtained from Sigma-Aldrich (Steinheim, Germany). Phenolic aldehydes —vanillin, syringaldehyde, coniferaldehyde, sinapaldehyde, *p*-hydroxybenzaldehyde, 3,4-dihydroxybenzaldehyde and 3,4,5-trihydroxybenzaldehyde—were used at concentrations levels of 0, 250 and 500 mg L^{-1}. Flavon-3-ols (quercetin, myricetin and kaempferol) were added at 0, 10, 20 and 40 mg L^{-1} and flavan-3-ols at 0, 12.5, 25 and 50 mg L^{-1} for (+)-catechin and at 3.12, 6.25 and 12.5 mg L^{-1} for (−)-epicatechin. The purity of the compounds was at least 97%, except for kaempferol (90%), myricetin (85%) and (−)-epicatechin (>90%). The disparity in the concentrations used was due to different levels of solubility of the flavonoids in the culture medium used. Fresh concentrated solutions of these compounds were prepared in pure (99.5%, v/v) ethanol and added to the growth media and the final ethanol concentration was adjusted to 5% (v/v). Each individual assay was made in triplicate and incubated aerobically, without agitation at 25 °C. The whole experiment was repeated to verify the results.

Bacterial growth was determined by measuring the culture medium absorbance at 660 nm, using an UV/vis UNICAM 8620 spectrophotometer (UNICAM, Cambridge, UK).

Inuence of tannins on the survival of O. oeni and Lact. hilgardii

Condensed tannins used were extracted from *Vitis vinifera* grape seed tissues, according to the method described by de Freitas et al. (2003). Polyphenols were extracted from grape seed tissues (5 g) with 50 mL of an ethanol/water/chloroform solution (1:1:2, v/v/v) using a blender. The upper aqueous layer containing the polyphenols was separated from the chloroform layer containing chlorophylls, lipids and other undesirable compounds. Ethanol was removed from the hydroalcoholic layer on a rotary evaporator at 30 °C and polyphenol compounds were extracted from the resulting aqueous solution with ethyl acetate (3 × 20 mL). The volume of ethyl acetate was reduced to 10 mL on a rotary evaporator at 30 °C and the condensed tannins were obtained by precipitation with 15 mL of hexane. The resulting solid was purified by column chromatography (TSK Toyopearl HW-40(s) gel) to yield catechin monomers and polymeric procyanidin fractions. These fractions were directly analysed by LSI/MS. The composition of the different fractions is as follows: Fraction A: catechins ($[M+H]^+$ at $m/z = 291$), catechins-gallate (443) and dimers (579); fraction B: dimers-gallate (579, 731), trimers (867), trimers-gallate (1019) and tetramers (1155); fraction C: tetramers-gallate (731, 867, 1019, 1155, 1307) and pentamers (1443); fraction D: tetramers-digallate (1459) and pentamers-gallate (1595).

The effect of tannins on the inactivation of *O. oeni* VF and *Lact. hilgardii* 5 was tested in a phosphate buffer solution (KH_2PO_4, 0.15 M, pH 4.5) with 10% (v/v) ethanol. Cultures in stationary phase, grown aerobically without agitation for 4 days in MRS/TJ with 5% (v/v) ethanol at 25 °C, were centrifuged (10 min, 3000*g*). The pellet was washed with phosphate buffer containing 5% (v/v) ethanol and centrifuged again. Cells were resuspended in phosphate buffer containing 5% (v/v) ethanol. One millilitre of this suspension was transferred to 100 mL of phosphate buffer (1% inoculum) containing 10% (v/v) ethanol and the tannin fraction at 500 mg L^{-1}, in 250 mL Erlenmeyer flasks. These were stirred magnetically and immersed in a thermostatted water bath at 25 °C. Two flasks were used as controls, one containing 100 mL of phosphate buffer and the other containing the buffer with added 10% (v/v) of ethanol. After the inoculation of the phosphate buffer, samples were collected at 5 s, 5, 15, 30, 45 and 60 min, properly diluted and plated in duplicate on MRS/TJ media containing 20.0 g L^{-1} Agar MC2 (LAB M, Bury, UK) and 5% (v/v) ethanol. Plates were incubated aerobically at 25 °C for 5–7 days. The whole experiment was repeated for each fraction of tannins and bacterium, to confirm the results.

Statistical analysis

All bacterial growth curves were analysed by non-linear regression (fitting Gompertz equation) for the independent estimation of growth parameters (μ, maximum growth rate; OD_{max}, maximum cell density). One-way analysis of variance (ANOVA) was used to test the effect of phenolic compounds' concentration on growth parameters, and *post hoc* Tukey test was used for multiple mean comparisons. All statistical analyses were performed with Prism 4 vs. 4.0a (GraphPad Software Inc.).

Results

Effect of phenolic aldehydes on the growth of O. oeni and Lact. hilgardii

At the levels normally found in wines (1–2 mg L^{-1}), phenolic aldehydes did not exert effect on the growth of the microorganisms studied (data not shown). The effect of these compounds were then studied at higher concentrations. From the phenolic aldehydes tested it can be seen that sinapaldehyde, coniferaldehyde, *p*-hydroxybenzaldehyde, 3,4-dihydroxybenzaldehyde and 3,4,5-trihydroxybenzaldehyde inhibited the growth of *O. oeni* VF (Fig. 2).

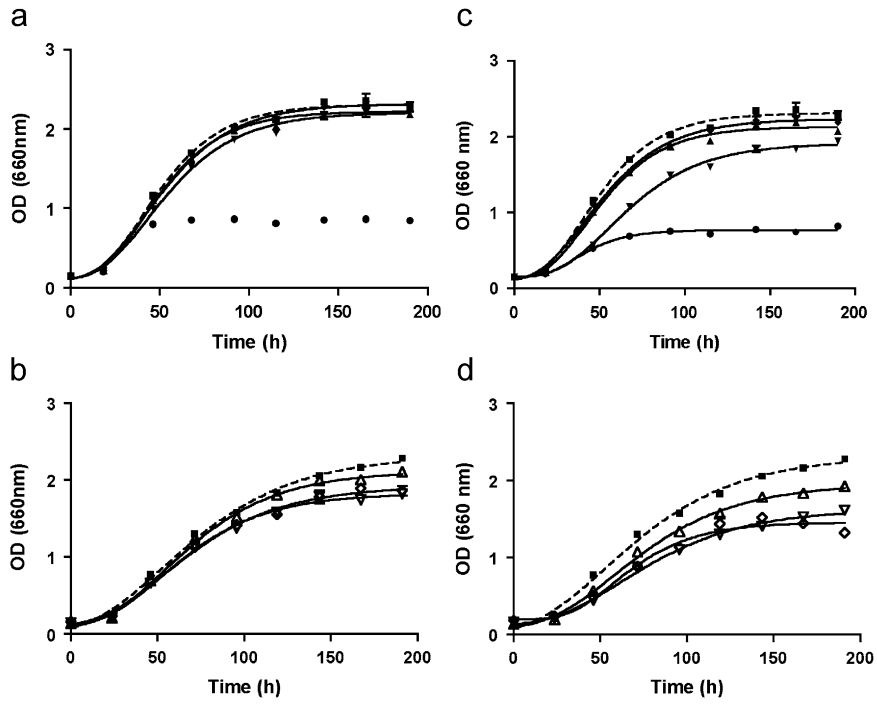

Fig. 2. Growth curves of *Oenococcus oeni* VF in MRS/TJ media (pH 4.5, 5% (v/v) ethanol at 25 °C) supplemented with phenolic aldehydes at (a and b) 250 mg L^{-1}, (c and d) 500 mg L^{-1}. (■) Control, (▲)vanillin, (♦) syringaldehyde, (▼) coniferaldehyde, (●) sinapaldehyde, (△) *p*-hydroxybenzaldehyde, (▽) 3,4-dihydroxybenzaldehyde, (◇) 3,4,5-trihydroxybenzaldehyde. Each point represents the average value of three determinations; vertical bars represent standard deviation for each set of determinations.

The highest effect was that of sinapaldehyde, clearly observed in both concentrations used, with a strong impact on the final cell density ($P<0.001$). The fitted line was omitted from the graph due to the poor fitting obtained with Gompertz equation. As we can see from data distribution, this fact was probably caused by the short number of empirical values on the initial range of the exponential growth phase. The effect of coniferaldehyde was significant only at 500 mg L^{-1} ($P<0.001$). The effect of the other phenolic aldehydes is also dose dependent and caused a significant decrease in the final cell density when compared to the control culture ($P<0.01$). Vanillin and syringaldehyde did not affect the growth of this bacterium at the concentrations tested (Fig. 2).

A decrease in the growth rate of *Lact. hilgardii* 5 in the presence of sinapaldehyde ($P<0.01$) and coniferaldehyde ($P<0.001$) was detected when the compounds were tested at 500 mg L^{-1} (Fig. 3c and d). This strain was not significantly affected by the presence of the other phenolic aldehydes tested.

Effect of flavonoids on the growth of O. oeni and Lact. hilgardii

The flavon-3-ols quercetin and kaempferol exerted a strong inhibitory dose-dependent effect on the growth of *O. oeni* VF (Fig. 4a–c). A marked decrease of the growth rate ($P<0.001$ for quercetin; $P<0.01$ for kaempferol) and of the final biomass ($P<0.001$) was obtained. Myricetin had no noticeable effect on the growth of this strain.

No significant effect was observed in the growth of *Lact. hilgardii* 5 in the presence of flavon-3-ols at 10 and 20 mg L^{-1} (Fig. 4d and e). At 40 mg L^{-1}, quercetin and kaempferol significantly decreased the growth rate ($P<0.05$) and increased the final cell density ($P<0.001$) (Fig. 4f).

None of the flavan-3-ols tested in this experiment (catechin and epicatechin) significantly affected the growth of *O. oeni* VF or *Lact. hilgardii* 5 (data not shown).

Inactivation of O. oeni and Lact. hilgardii by tannins

The influence of tannins on cell physiology was studied by the progressive inactivation of cells in ethanol-containing phosphate buffer supplemented with different tannin fractions. Growth assays were not carried out since the tannins were found to be difficult to solubilise in the culture medium probably due to the interaction with proteins. All fractions of tannins tested led to a marked decrease in the number of viable cells of *O. oeni* VF, causing a reduction of 4 log cycles or more (Fig. 5a). It was also noted that the inactivation effect increases with the increment of the average tannin size in the fractions (from A to C). A similar behaviour was found for *Lact. hilgardii* 5 but with a milder effect. Fraction A did not show a noticeable inactivation and fraction C caused a 2.5 log cycles reduction in 60 min (Fig. 5b). In both strains the highest effect was obtained when cells were treated with fraction C. The control experiment performed in buffer with 10% (v/v) ethanol did not affect the survival of both strains. Cells exposed to the

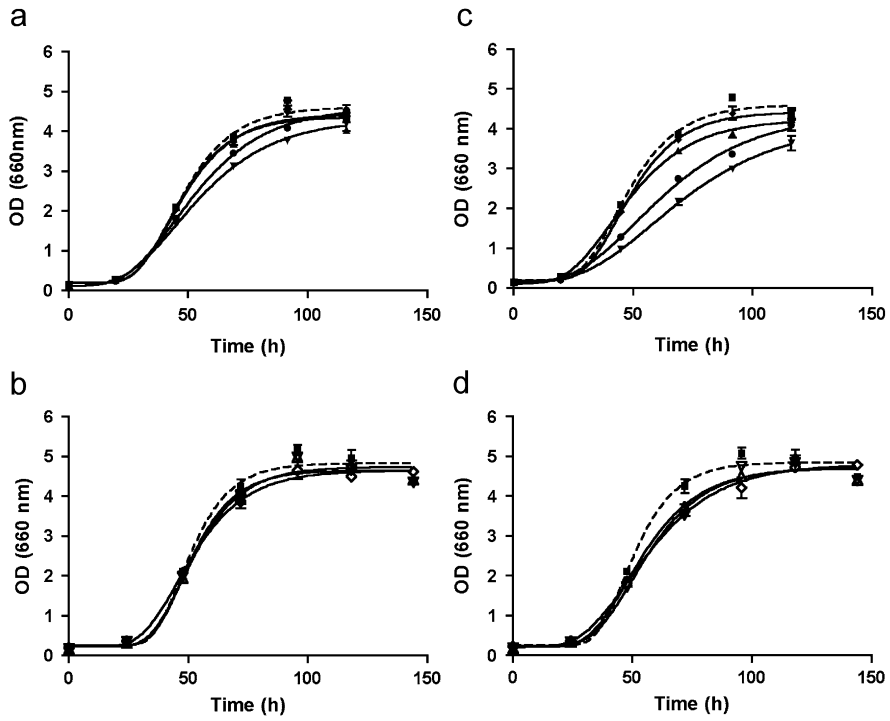

Fig. 3. Growth curves of *Lactobacillus hilgardii* 5 in MRS/TJ media (pH 4.5, 5% (v/v) ethanol at 25 °C) supplemented with phenolic aldehydes at (a and b) 250 mg L^{-1}, (c and d) 500 mg L^{-1}. (■) Control, (▲)vanillin, (♦) syringaldehyde, (▼) coniferaldehyde, (●) sinapaldehyde, (△) *p*-hydroxybenzaldehyde, (▽) 3,4-dihydroxybenzaldehyde, (◇) 3,4,5-trihydroxybenzaldehyde. Each point represents the average value of three determinations; vertical bars represent standard deviation for each set of determinations.

different tannin fractions were observed under the light microscope after 30 and 60 min of exposure. Clumping effects were not observed in both strains for the four tannin fractions experimented. Cells were found to be homogeneously distributed in the liquid in a similar manner as the control culture.

Discussion

This work provides insight into the effect of various phenolic compounds on wine lactic acid bacteria. Most of the phenolic aldehydes tested influenced the growth of *O. oeni* VF. Cinnamaldehydes (particularly sinapaldehyde) were the ones that most affected growth of this strain by diminishing the growth rate and reducing the final cellular concentration. This inhibitory effect, although less strong, was also observed for some benzaldehydes (*p*-hydroxybenzaldehyde, 3,4-dihydroxybenzaldehyde and 3,4,5-trihydroxybenzaldehyde). The growth rate of *Lact. hilgardii* 5 was negatively influenced only by cinnamaldehydes. These results are in accordance with the previous work done by Campos et al. (2003) with structurally similar phenolic (cinnamic and benzoic) acids that only differ from the tested aldehydes in the functional group. The less polar nature of the cinnamaldehydes, due to their propionic side chain, might confer a higher affinity to the lipid content of the bacterial cell membrane (O'Connor and Rubino, 1991), possibly enabling these molecules to move more readily across the outer cell layers. Although several studies already identified cinnamaldehydes as bactericidal agents (Kwon et al., 2003; Gill and Holley, 2004; Kim et al., 2004), their mechanism of action is not yet fully understood and probably varies according to the microorganism. For instance, in *Bacillus cereus* cinnamaldehyde does not exhibit cell lysis but affect cell viability (Kwon et al., 2003), while in *Listeria monocytogenes* and *Escherichia coli* cell membrane disruption was advanced as a possible mechanism of cinnamaldehyde action, dispersing the proton motive force by leakage of small ions (Gill and Holley, 2004; Kim et al., 2004). The number of hydroxyl groups in the benzaldehyde derivates that inhibited the growth of *O. oeni* VF seems to be related to their inhibitory power. The disubstituted and trisubstituted benzaldehydes had a higher inhibitory effect than *p*-hydroxybenzaldehyde. Benzaldehydes are thought to act primarily on the external surface of the cells, combining with sulphydryl groups of proteins (Ramos-Nino et al., 1998). Ramos-Nino et al. (1996) and Friedman et al. (2003) reported that benzaldehydes with two or more adjacent hydroxyl groups are more active than aldehydes that lack this characteristic, which is in agreement with our results. At the concentrations used in this work (higher than those normally found in wines), vanillin did not affect the growth of both strains. At higher concentrations, however, Fitzgerald et al. (2004) found that vanillin may have antimicrobial activity against *E. coli*, *Listeria innocua* and *Lactobacillus plantarum*. According to these authors, vanillin affects the integrity of the cytoplasmic membrane causing loss of ion gradients and pH

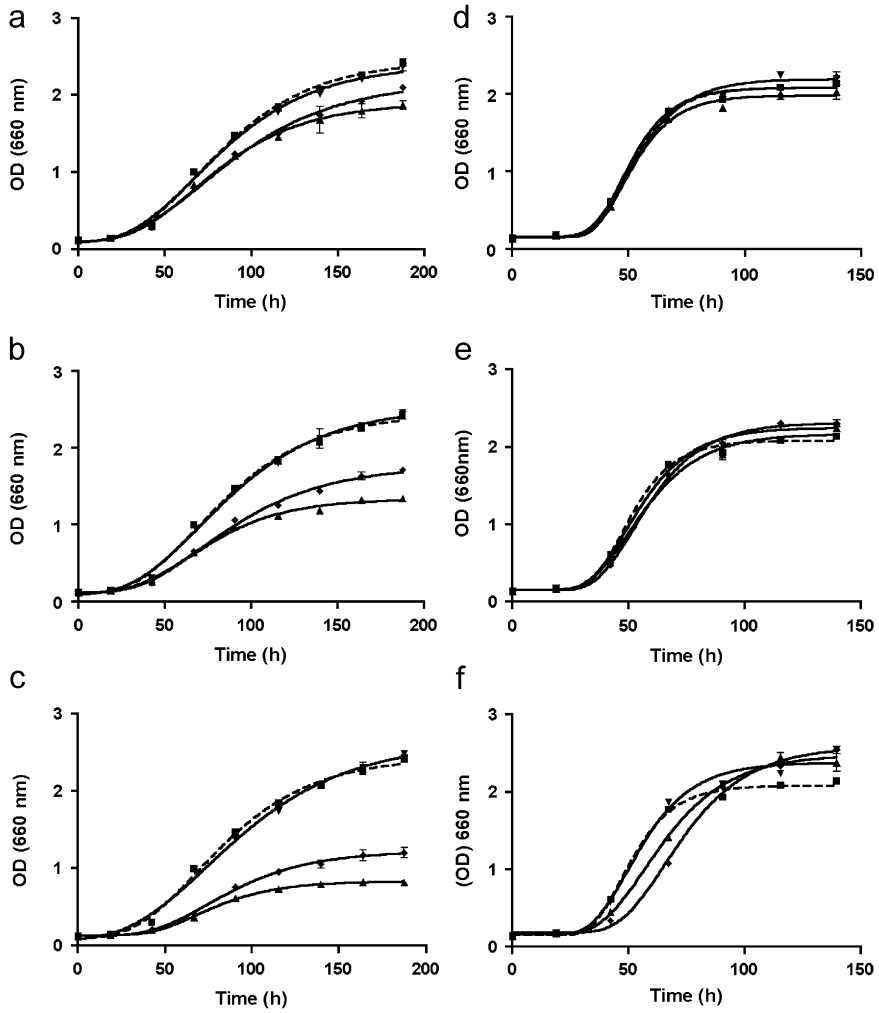

Fig. 4. Growth curves of *Oenococcus oeni* VF (a–c) and *Lactobacillus hilgardii* 5 (d–f) in MRS/TJ media (pH 4.5, 5% (v/v) ethanol at 25 °C) supplemented with (a and d) 10 mg L^{-1}, (b and e) 20 mg L^{-1}, (c and f) 40 mg L^{-1}. (■) Control, (▲) quercetin, (▼) myricetin and (♦) kaempferol. Each point represents the average value of three determinations; vertical bars represent standard deviation for each set of determinations.

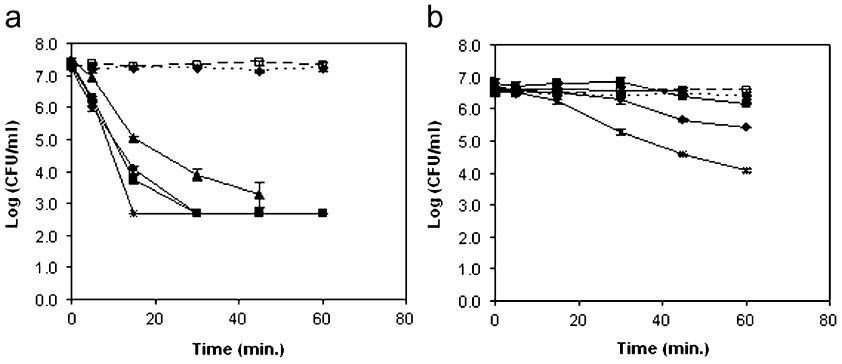

Fig. 5. Inactivation curves of (a) *Oenococcus oeni* VF and (b) *Lactobacillus hilgardii* 5 in phosphate buffer (pH 4.5, 10% (v/v) ethanol) at 25 °C, supplemented with tannins at 500 ppm: (♦) control, (□) control with 10% ethanol, (▲) fraction A, (■) fraction B, (✳) fraction C and (●) fraction D. Each point represents the average value of three determinations, except for fraction A, where each point represents the average value of two determinations; vertical bars represent standard deviation for each set of determinations.

homeostasis. However, this effect was non-lethal and depends on the time of exposure, concentration and target organism.

Of the three flavon-3-ols tested, only the ones harbouring B-rings with less hydroxyl substitutions, kaempferol and quercetin, inhibited the growth of *O. oeni* VF. Myricetin

(with three OH substitutions) did not influence the growth of this strain at the tested concentration levels. In the case of *Lact. hilgardii* 5, only the highest concentration (40 mg L^{-1}) of quercetin and kaempferol slightly decreased the growth rate (the concentration of these compounds in red wine are in the region of 100 mg L^{-1}; Ribéreau-Gayon et al., 2000). Unexpectedly, at the mentioned concentration, the final cell density attained in the presence of the flavon-3-ols, especially quercetin, was higher than that of the control. Flavon-3-ols are known to possess antioxidant properties by acting as oxygen free radical scavengers. This ability is related to the B-ring hydroxyl configuration and is especially enhanced by the presence of a catechol structure in this ring (Heim et al., 2002). Thus, a possible antioxidant effect could be responsible for the extended growth of *Lact. hilgardii* 5 in the presence of flavon-3-ols. Our results indicate that flavon-3-ol toxicity cannot be directly related to the degree of hydroxylation (and hence, to the polarity) of the B-ring. This could mean that the inhibitory action of these flavonoids could occur from the extracellular environment or that it could be limited by their ability to cross the cell membrane. A similar result was obtained in previous experiments done with phenolic acids (Campos et al., 2003), which suggested that the polarity of the molecules could not explain, by itself, the observed differences in toxicity of these compounds. Quercetin is reported to have antibacterial properties either by inhibiting DNA replication or by acting at the cell membrane level (Cuhsnie and Lambert, 2005). On the other hand, Padmavati et al. (1997) suggested that lipophilicity could be an important factor in flavonoid toxicity. In accordance to this, Puupponen-Pimiä et al. (2001) compared different flavon-3-ols and reported myricetin as being the only inhibitory compound towards lactic acid bacteria from the human gastrointestinal tract flora.

Flavan-3-ols did not affect the growth of the studied bacteria at the concentrations tested, which are within or very close to the levels found in wine (10–200 mg L^{-1}) (Goldberg et al., 1998). It is known that catechin can stimulate the growth of some strains of *Lact. hilgardii* at the concentrations normally present in wine (Alberto et al., 2001). This stimulation was explained with the ability of these strains to metabolize these classes of phenolic compounds. The absence of stimulus on the growth of the strain used in this work suggests that this ability could be strain dependent. Previous studies also demonstrated that catechin does not have any effect on the growth of several bacterial species (Rauha et al., 2000), including lactic acid bacteria (Puupponen-Pimiä et al., 2001).

The influence of grape seed tannins was evaluated by studying its effect on cell viability in non-growing conditions. It is often claimed that tannins have antiseptic properties, but the scientific data supporting this assertion is very scarce. Our results indicate that some tannin fractions may exert a significant antimicrobial action, clearly seen even at the concentration of 0.5 g L^{-1}, which is below the normal level of condensed tannins dissolved in wine (1–4 g L^{-1}, depending on grape variety and wine-making procedures; Ribéreau-Gayon et al., 2000). As mentioned above, the condensed tannins' structural units (catechin and epicatechin) did not influence bacterial growth, while it was found that oligomeric and polymeric forms of these compounds exhibit toxic capacities. It is evident, however, that strains of bacteria differ considerably in their tolerance towards tannins, *O. oeni* VF being much more affected than *Lact. hilgardii* 5. The results obtained from the viability experiments in non-growing conditions, strongly suggest that the influence of tannins is due to a direct effect on the organisms rather than by the inactivation/precipitation of any essential nutrients. Taking into account their high molecular weight, it might be expected that tannins exert its influence from the extracellular medium. Tannins can bind to membrane phospholipids and proteins disrupting membrane function (Jackson, 2000) or may establish hydrogen links with the peptidoglycan of Gram-positive bacteria cell wall (Field and Lettinga, 1992). In this work, it is perceptible that cell viability decreased with the increment of the tannins size until fraction C, composed by tetramers-gallate and pentamer macromolecules. The effect of fraction D was less pronounced than fraction C and similar to fraction B. The results suggest that, to a certain limit, the higher the molecular weight of tannins the higher is its interaction with the cell surface and thus the higher is its effect.

In conclusion, it is demonstrated that different classes of wine phenolic compounds interact with wine lactic acid bacteria. Besides the very well-known effect of parameters like pH, ethanol and sulphur dioxide on microorganisms, the action of phenolic compounds should be considered as a meaningful factor which may influence the growth and activity of lactic acid bacteria in wine. The present work shows that phenolic compounds essentially exhibit microbial inhibitory properties, which may influence the onset and/or the terminus of the malolactic fermentation. Further research is needed to assess whether the phenolic compounds studied also inhibit lactic acid bacteria in real wine conditions.

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