Cell Membrane Damage Induced by Phenolic Acids on Wine Lactic Acid Bacteria

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Abstract

Phenolic compounds are known to have membrane-active properties against microorganisms causing leakage of cell constituents. In this work, several phenolic acids were tested for their effects on the cell membrane of wine lactic acid bacteria (*Oenococcus oeni* and *Lactobacillus hilgardii*) by measuring potassium and phosphate leakage from the cells. The experimental results obtained indicate that *p*-coumaric acid had the strongest effect of all tested compounds, causing rapid ion leakages from cells. Generally, hydroxycinnamic acids induced faster leakage rates than hydroxybenzoic acids, which could be related to their higher lipophilic character. These results agree with previous results obtained in growth and inactivation experiments with the same strains.

Introduction

Lactic acid bacteria play an important role in wine quality, being responsible for the occurrence of malolactic fermentation (which occurs in most red wines). *Oenococcus oeni* is the main bacteria responsible for this process and is commonly used as starter culture. Other lactic acid bacteria, like *Lactobacillus hilgardii* (commonly found in spoiled fortified wines), can cause wine deterioration, increasing its volatile acidity and producing off-flavours.

It has been empirically known for years that the phenolic content of grapes and wines can affect the rate of the malolactic fermentation. The phenolic composition of wines is much diversified and includes phenolic (hydroxybenzoic and hydroxycinnamic acids) in concentrations up to 200 mg L^{-1} [1]. Despite their structural similarities (see Fig. 1), phenolic acids have different effects on growth and inactivation of wine lactic bacteria [2]. Phenolic compounds are known to have membrane-active properties against microorganisms [3]. These compounds diffuse through the cytoplasmic membrane increasing its permeability. Following their influx there is usually a leakage of bacterial cell constituents including proteins, nucleic acids, and inorganic ions such as potassium or phosphate. The measurement of the efflux rates of these ions (using potentiometric or photometric methods) has been used previously to monitor cell membrane damage [4]. In this work, several wine-occurring phenolic acids were tested for their effects on the cell membrane integrity of *O. oeni* VF (a commercial starter culture strain) and *Lact. hilgardii* 5 (a wild-type strain isolated from spoiled Port wine).

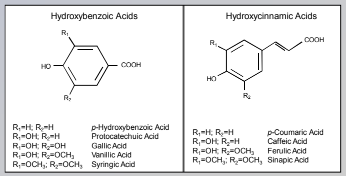

Figure 1 – Structural formulae of wine phenolic acids

Monitoring systems

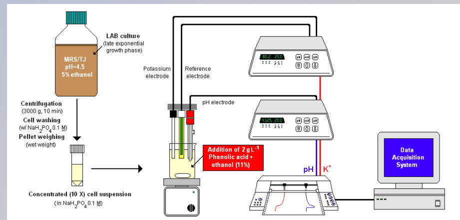

Figure 2 – Potassium efflux monitoring system. Extracellular pH and potassium concentration measurements were made using two potentiometers connected to a data acquisition system.

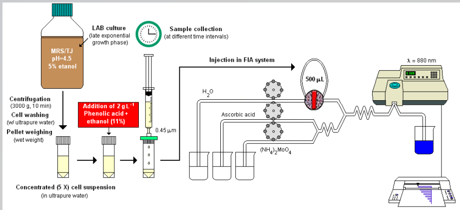

Figure 3 – Phosphate efflux monitoring system. Samples were collected, microfiltered and injected in the FIA system depicted above. Phosphate concentration was determined spectrophotometrically using a reaction with ammonium molybdate and ascorbic acid.

Results

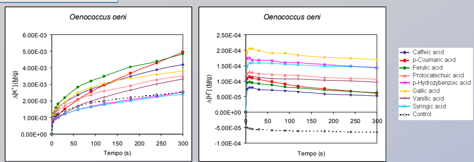

Figure 4 – Variation of the extracellular K^+ and H^+ concentrations of a *Oenococcus oeni* VF suspension exposed to chemical stress with different phenolic acids (at 2,2 g/L) in phosphate buffer with 11% v/v ethanol; values corrected for cellular wet weight.

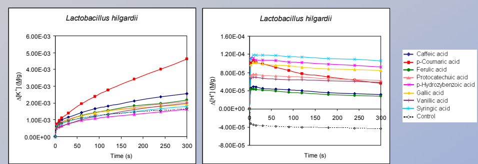

Figure 5 – Variation of the extracellular K^+ and H^+ concentrations of a *Lactobacillus hilgardii* 5 suspension exposed to chemical stresses with different phenolic acids (at 2.2 g/L) in phosphate buffer with 11% v/v ethanol; values corrected for cell wet weight.

Results

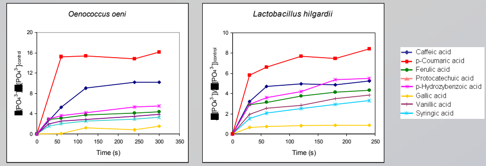

Figure 6 – Variation of the extracellular phosphate concentrations of *Oenococcus oeni* VF and *Lactobacillus hilgardii* 5 suspensions exposed to chemical stresses with different phenolic acids (at 2.0 g/L) in ultrapure water with 11% v/v ethanol; values corrected for cell wet weight and divided by the results obtained in the control assays (not shown).

Conclusions

- Hydroxycinnamic acids caused higher potassium and phosphate effluxes than hydroxybenzoic acids in both strains of lactic acid bacteria. *p*-Coumaric acid had the highest effect of all tested phenolic acids, causing rapid leakage of both inorganic ions.

- Results obtained with both efflux monitoring systems agree with previously published data obtained in growth and inactivation experiments performed with the same strains [2]. Efflux rates were higher in the case of the phenolic acids which had a more negative effect on growth and inactivation of these bacteria.

- pH variation of the extracellular medium was inversely proportional to potassium efflux which could be related to chemical equilibrium shifts caused by phenolic acids diffusing into the bacterial cells at different rates.

- The different effects of phenolic acids on membrane permeability could to be related to differences in their lipophilic character.

References

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