

Development of a flow method for monitoring cell membrane damage: application to the study of phosphate release from wine lactic acid bacteria



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Leakage of cellular metabolites like nucleotides, amino acids and inorganic ions, is a primary indication of cell membrane damage in bacteria that can be caused by chemical agents. The measurement of the efflux rates of phosphate ions (using photometric methods) can be used to monitor membrane damage and to compare the effects of different chemical compounds.

Phenolic compounds (Figure 1) 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 phosphate leakage from the cells via flow injection analysis.

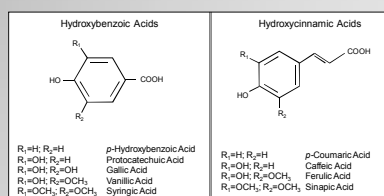


Figure 1. Structural formulae of wine phenolic acids

Flow injection manifold

The determination of phosphate efflux was based on the formation of phosphomolybdate heteropoly acid and on its spectrophotometric detection.

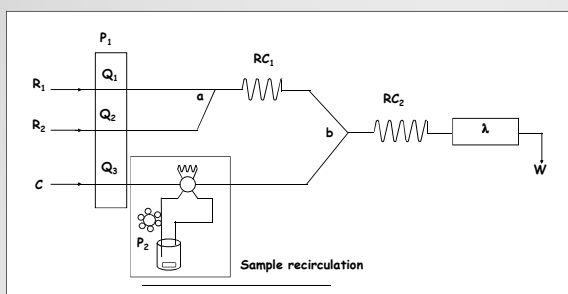


Figure 2. Flow injection manifold developed for the determination of phosphate leakage of LAB exposed to phenolic acids with a sample recirculation system; P₁ and P₂, peristaltic pumps; R₁ molybdate reagent; R₂, ascorbic acid; C, carrier; RC, reaction coils (RC₁, 100 cm; RC₂, 100 cm); Q flow rates (Q₁ = Q₂ = 1.9 mL min⁻¹; Q₃ = 4.0 mL min⁻¹); W, waste; a and b, confluence points; λ, UV/Vis spectrophotometer (880 nm).

Repeatability

Standards and cell suspensions	RSD (n=10)
6.45x10 ⁻⁵ mol P/L	1.4%
2.42x10 ⁻⁴ mol P/L	1.7%
4.03x10 ⁻⁴ mol P/L	1.6%
<i>O. oeni</i> VF	1.2%
<i>Lact. hilgardii</i> 5	2.9%

Application to the study of phosphate release:

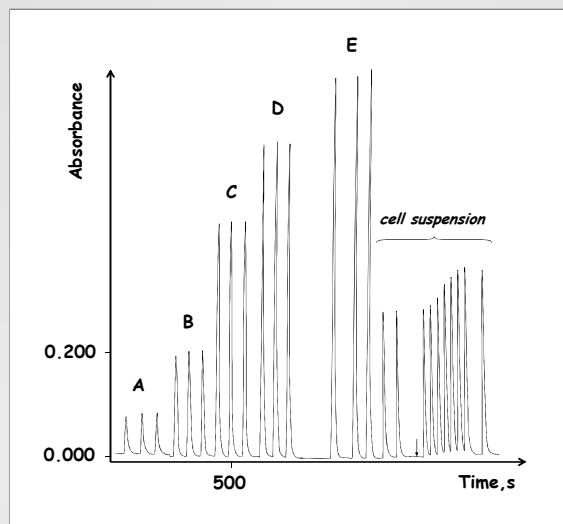


Figure 3. Recorder output for the determination of phosphate efflux corresponding to injections of phosphate standards: (A) 6.45x10⁻⁵ (B) 1.29x10⁻⁴ (C) 2.42x10⁻⁴ (D) 3.23x10⁻⁴ (E) 4.03x10⁻⁴ mol L⁻¹; and sample: *O. oeni* VF; arrow indicates the time of addition of caffeic acid.

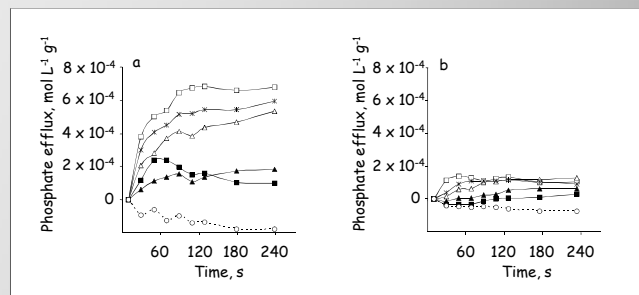


Figure 4. Phosphate efflux of *Lact. hilgardii* 5 (a) and *O. oeni* VF (b) suspensions after chemical stress with phenolic acids at 0.0122 mol L⁻¹ in ethanol. (□) *p*-coumaric acid, (■) *p*-hydroxybenzoic acid, (*) caffeic acid, (▲) vanillic acid, (△) ferulic acid, (○) control. Results are expressed as variation of phosphate concentration per wet weight of cellular pellet.

Conclusions

- The FIA system developed in this work proved to be a fast and reliable alternative method for comparing the effect of different compounds.
- Hydroxycinnamic acids have a stronger effect than hydroxybenzoic acids on cell permeabilization. These results agree with data obtained in growth and inactivation experiments performed with the same strains (Campos et al., 2003).
- The proposed approach might have an application in the food or pharmaceutical industries.

Reference

Campos FM, Couto JA, Hogg TA. 2003. Influence of phenolic acids on grow and inactivation of *Oenococcus oeni* and *Lactobacillus hilgardii*. J Appl Microbiol 94:167-174.