Influence of Phenolic Compounds on Glucose and Organic Acids Metabolism of Wine Lactic Acid Bacteria

Bilé T. I., Sá F. S., Campos F. M., Figueiredo A. R., Couto J. A. and Hogg T. A.

Escola Superior de Biotecnologia, Universidade Católica Portuguesa , Rua Dr. António Bernardino de Almeida, 4200-072 Porto, Portugal; E-mail: teresa.bile@hotmail.com

ABSTRACT

In this work we studied the influence of phenolic acids and flavonoids (flavan-3-ols and flavon-3-ols) on glucose and organic acid metabolism by wine lactic acid bacteria (*O. oeni* and *Lact. hilgardii*). Cultures were grown in MRS supplemented with L-malic acid (5.0 g/L) and different phenolic compounds. Cell growth was monitored spectrophotometrically and metabolite concentrations were determined by HPLC. Generally, the presence of phenolic acids in the growth medium delayed the consumption of glucose and organic acids by these bacteria. On the other hand, flavonoids apparently stimulated metabolism of these compounds suggesting that the presence of these compounds might accelerate the conclusion of malolactic fermentation.

INTRODUCTION

Lactic acid bacteria (LAB) can multiply in wines and metabolize residual sugars, organic acids and other metabolites affecting the quality of finished wines. These bacteria (particularly strains of *O. oeni*) are responsible for the occurrence of malolactic fermentation (the conversion of L-malic to L-lactic acid) which can have a positive impact in the organoleptic properties of some wines. On the other hand, LAB can also produce undesirable aromas and other defects at later stages of vinification, reducing wine quality [1]. *Lactobacillus hilgardii* is frequently associated with spoilage of sweet fortified wines (such as Port wine).

Some wines (particularly red ones) are rich in phenolic compounds extracted either from grape material or from oak wood. These compounds are responsible for important wine attributes such as colour, astringency and bitterness and have also some impact on wine *bouquet*. The phenolic composition of wines is very diversified and include phenolic acids and flavonoids at variable concentrations depending on grape variety and winemaking technology [2].

Several published works have shown that while some phenolic compounds have a negative effect on LAB growth and survival, others might actually stimulate their growth [3,4].

In this work we studied the influence of phenolic acids and flavonoids (flavan-3-ols and flavon-3-ols) on glucose and organic acid metabolism of two strains of heterofermentative lactic acid bacteria.

MATERIAL AND METHODS

Bacterial strains:

Lact. hilgardii 5 – originally isolated from Port wine (ESBUCP culture collection, Porto, Portugal)

O. oeni Viniflora Oenos – commercial starter culture (Christian Hansen, Hrevidre, Denmark)

Growth conditions:

Growth medium: MRS (de Man, Rogosa and Sharpe), pH=4.5, 5% ethanol supplemented with 5 g L^{-1} L-malic acid; MRS contained approximately 2 g L^{-1} ammonium citrate and 5 g L^{-1} potassium acetate. Pre-cultures were grown in MRS/Tomato Juice (50:50) to late exponential phase (3-4 days). All cultures were incubated at 25ºC in aerobiosis.

Phenolic compounds tested:

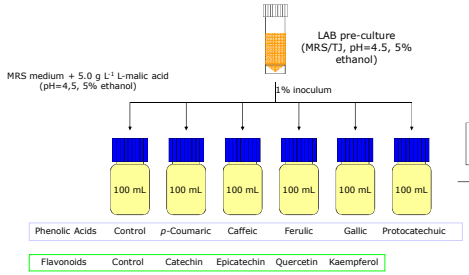
Phenolic acids: *p*-coumaric, caffeic, ferulic, gallic and protocatechuic acids (at 500 mg L^{-1})

Flavan-3-ols: (+)-catechin and (-)-epicatechin (at 100 mg L^{-1})

Flavon-3-ols: quercetin and kaempferol (at 40 mg L^{-1})

Growth monitoring and HPLC analysis

Samples were collected daily to monitor cell growth and were frozen (at -20ºC) for later analysis. Cell growth was monitored spectrophotometrically (λ=660 nm) and glucose and organic acids (malic, lactic and acetic) concentrations were determined by HPLC using the operation conditions described below.



HPLC system	BECKMAN GOLD (w/ Refractive Index detector)
Column	BIORAD AMINEX HPX-87 H (300 x 7.8 mm)
Temperature	50 ºC
Mobile Phase	H_2SO_4 2.5 mM, 0.6 mL min^{-1}
Injection volume	20 mL
Sample treatment	Microfiltration (0.45 mm)

RESULTS

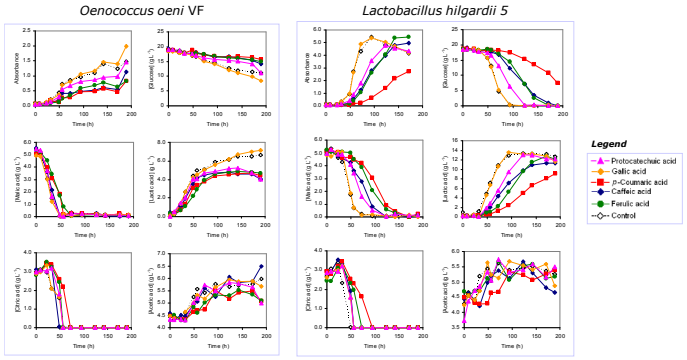


Figure 1 – Growth and metabolism of *O. oeni* VF and *Lact. hilgardii* 5 in MRS medium supplemented with 5 g L^{-1} L-malic acid and phenolic acids (500 mg L^{-1})

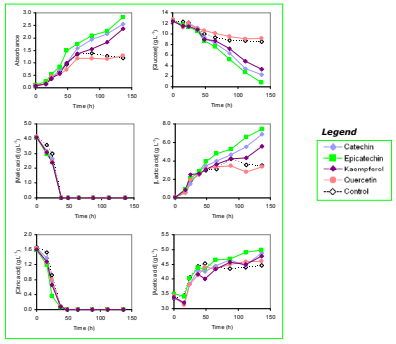


Figure 2 – Growth and metabolism of *O. oeni* VF in MRS medium supplemented with 5 g L^{-1} L-malic acid and flavonoids.

DISCUSSION

- Phenolic acids (except for gallic acid) had a negative effect on growth and glucose metabolism of *O. oeni* VF. On the contrary, flavonoids (except for quercetin) had the opposite effect, extending growth and glucose metabolism of this bacteria comparatively to the control.
- Apparently, the malolactic activity of *O. oeni* VF was not affected by the presence of phenolic acids or flavonoids in the growth medium.
- In the case of *Lact. hilgardii* 5, phenolic acids delayed growth and consumption of glucose and organic (citric and malic) acids. The presence of flavonoids in the growth medium did not seem to affect this strain considerably (results not shown).
- The results obtained suggest that some flavonoids (catechin, epicatechin, kaempferol) can stimulate growth and the glucose metabolism of *O. oeni*. This effect could be related to the antioxidant properties of these compounds.

REFERENCES

[1] Davis, C.R., D.J., Lee, T.H. and Fleet, G.H. (1986), *Applied and Environmental Microbiology*, 51 (3), 539-545

[2] Reguant, C., Bordons, A., Arola, L. and Rozès, N. (2000), *Journal of Applied Microbiology* 88, 1065-1071

[3] Campos, F.M., Couto, J.A. and Hogg, T.A. (2003), *Journal of Applied Microbiology* 94, 167-174.

[4] Vivas, N., Lonvaud-Funel, A. and Glories, Y. (1997), *Food Microbiology*, 14, 291-300

ACKNOWLEDGMENTS

The authors would like to thank FCT (Fundação para a Ciência e a Tecnologia) for funding this research via project POCTI/AGG/40724/2001. F. M. Campos would also like to thank FCT for the grant PRAXIS XXI/BD/19909/99. This research was funded by the European Social Fund (III Community Support Framework).