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ORIGINAL ARTICLE

Effect of phenolic acids and anthocyanins on growth, viability and malolactic activity of a lactic acid bacterium

N. Vivas[1]*, A. Lonvaud-Funel[2] and Y. Glories[2]

Phenolic compounds are important components of red wine and potentially affect malolactic fermentation. Growth and metabolism of Leuconostoc oenos *may be influenced favourably or unfavourably. The effect of phenolic acids and free anthocyanins was studied. Gallic acid and free anthocyanins activated cell growth and the rate of malic acid degradation. Vanillic acid showed a slight inhibiting effect, while protocatechuic acid had no effect. Finally gallic acid and anthocyanins were metabolized, specially by growing cells.*

Introduction

Lactic acid bacteria, particularly *Leuconostoc oenos*, are responsible for the transformation of malic acid into lactic acid during vinification (malolactic fermentation). This was shown by Peynaud (1967) in the first published results on the lactic acid microflora of grape must and wines. After the works of Ribereau-Gayon et al. (1975), this second phase of vinification has been intensively studied worldwide by numerous authors (Lonvaud-Funel 1994). Today enologists recommend malolactic fermentation (MLF) in most red wines and some white wines, particularly in those wines where ageing in oak barrels and long-time maturation in bottles are a part of the process.

Alcoholic fermentation has become increasingly well-regulated but MLF still poses problems. Many papers relate the effects of temperature, ethanol, pH and sulfur dioxide on growth and malolactic activity of *L. oenos* (Lafon-Lafourcade 1973). These four parameters determine the beginning and the rate of MLF (Ribereau-Gayon et al. 1975; Kunkee 1991). More recently, attention was focused on other complications of MLF in wines in relation to microbial interaction. Beelman et al. (1982) described the inhibitory effect of yeast on bacterial growth and Lonvaud-Funel et al. (1988) identified fatty acids as the main inhibitory products of yeast metabolism. Antagonisms were also demonstrated between strains of lactic acid bacteria (Lonvaud-Funel and Joyeux 1993). However, wine is conducive to MLF, otherwise it would be impossible; it is spontaneous in most cases. Wine contains the nutritional requirements needed for MLF and provided the addition of L-malic acid, and possibly citric acid, it can be induced several times in the same wine by successive inoculations (Lonvaud-Funel 1986). Moreover only very low amounts of sugars (glucose, fructose,

*Corresponding author: N. Vivas, Faculté d'OEnologie, 351 cours de la libération, F-33405 Talence.

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[1]*Tonnellerie DEMPTOS S.A. posted to Faculté d'OEnologie, Université de Bordeaux II, 351 cours de la libération, 33405 Talence, France*
[2]*Faculté d'OEnologie, Université de Bordeaux II, 351 cours de la libération, 33405 Talence, France*

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arabinose, xylose, galactose) less than 200–300 mg l^{-1} are used during the growth of the lactic acid bacteria. The process is easier in red wines compared with white wines under the same principal conditions (pH, ethanol, temperature, total SO_2, L-malic acid).

Red wines contain large amounts of phenolic compounds: phenolic acids, anthocyanins, flavonols and tannins. Tannins and phenols may be used by bacteria; this has been widely studied in environmental detoxification. These compounds may affect growth and metabolism of bacteria (Scalbert 1992). In wines, one of the first studies dealing with this subject, was done by Saraïva (1983). The author shows the inhibiting effect of phenolic acids and tannins on the growth of lactic acid bacteria and the activating role of anthocyanins. Also phenolic compounds affect whether or not MLF will occur and the rate of MLF.

In this work we study the influence of some phenolic acids and grape-skin anthocyanins on growth and malolactic activity of *L. oenos*.

Materials and Methods

Origins of phenolics compounds

Three phenolic acids of the benzoic series (Extrasynthese™), were chosen for their different phenolic substitution protocatechuic acid (2 OH in *ortho* position), gallic acid (3 OH in *ortho* position) and vanillic acid (1 OH and 1 OCH_3 in *ortho* position).

Grape-skin anthocyanins: 100 g of skins of mature Cabernet Sauvignon (1992, Bordeaux area) were extracted for 12 h in 500 ml alcoholic solution (69:30:1, EtOH:H_2O:HCl N (v:v:v) on a rotative table at room temperature (20±1°C). The hydroalcoholic extract was washed by 2×100 ml hexane for eliminating lipids and fatty acids. Free anthocyanins, according to Glories's definition (1978, 1984), were isolated by chromatography on a polyvinyl-polypyrrolidon column (pvpp, Touzart–Matignon™, column dimension: 7 cm×60 cm). Preparation and purification of insoluble PVPP was done according to the method of Glories (1976). The extracts containing the free anthocyanins were concentrated to 50 ml using a rotary evaporator at 25°C. They were loaded on a column previously washed with 500 ml water; the free anthocyanins were collected after elution with 500 ml of 'Ha' solvent (70:30:1, EtOH:H_2O:HCl), the fraction was evaporated using a rotary evaporator and lyophilized. This powder contained 92% (w:w) free anthocyanins, 8% (w:w) different phenolic acids and traces of flavanols. The composition of the anthocyanin fraction was: monoglucoside-free anthocyanins (mG) 54% (7·5% delphinidin-3-mG, 1% cyanidin-3-mG, 8% petunidin-3-mG, 5·5% paeonidin-3-mG, 32% malvidin-3-mG) and acylated free anthocyanins 46% (mGA).

Pure malvidin-3-mG and its aglucon malvidin were provided by Extrasynthese™.

Bacterial strains and growth medium

The following strain was used: *L. oenos* IB8413 isolated from a red wine (collection of the Faculté d'OEnologie, Bordeaux, France). It was grown in the Carr medium (Carr 1956) containing (per liter): 5 g casamino acids (Difco), 4 g yeast extract (Difco), 5 g glucose, 10 g D–L malic acid, 0·025 g $MnSO_4$, H_2O, 0·125 g $MgSO_4$, $4H_2O$, 0·425 g KCl, 0·55 g KH_2PO_4, 0·125 g $CaCl_2$, 1 ml Tween 80, adjusted to pH 4·8. Decolorized wine: a young red wine of Cabernet Sauvignon (1992, Bordeaux area) containing 2·85 g l^{-1} malic acid was treated 8 g l^{-1} charcoal, and sterilized by filtration (pore size 0·45 μm).

Growth measurements

Growth of lactic acid bacteria was followed by plate counts. Carr medium supplemented with tomato juice, was added together with 250 ml l^{-1}; 20 g gelose (Difco), pimaricine, 20 ml of a solution at 5 mg ml^{-1}. Incubation was conducted in anaerobiosis (Queue incubator, CO_2/N_2, 20:80, v:v) at 25°C for 5 days.

Viability of resting cells

The biomass (10^9 cfu ml^{-1}) was obtained by centrifugation (10 min, 10 000 ***g***, 4°C) of a stationary phase culture in Carr medium.

Cells were washed with phosphate buffer (KH_2PO_4, 0·15 M, pH 4·5) and centrifuged. Viability was studied at 25°C in phosphate buffer with various concentrations of phenolic compounds added. Survival was expressed as log N_t/logN_o where N_o was the number of cfu ml^{-1} in the control medium and N_t in the medium with different phenolic compounds added after 48h.

Determination of malic acid

Malic acid determination was performed using an enzymatic test kit (Boehringer-Mannheim™).

Determination of malolactic activity

Malolactic activity of resting cells was estimated with P_{CO_2} electrode according to the method of Lonvaud (1975).

Estimation and determination of phenolic compounds

Estimation of phenolic acids was made by measurement of optical density (OD) at 280 nm (Spectrophotometer Beckman™, D.U. 64); the culture medium without any phenolic compound acted as the control. The specific evolution of the various phenolic acids was followed by thin layer chromatography (TLC). Samples (2 µl) were sprayed on TLC plates (RP18, WF254, Merck™, 10×10×0·2 cm) with Camag™ automated deposit (N_2 as propulsing gas; spot dimension: 5 mm). Migration was performed in a horizontal migration chamber at room temperature (10°C) using MeOH:H_2O:HCOOH as the solvent (50:40:10, v:v:v). The spectra of separated products of the plates were read at 280 nm with a densitometer Camag™. In these conditions the R_f of the phenolic acids were: gallic acid, 0·68; protocatechuic acid, 0·55; vanillic acid, 0·36. The different compounds were identified by UV spectra (220–400 nm).

Total free anthocyanins (mG+mGA) were determined by the method of Ribereau-Gayon and Stonestreet (1966). Malvidine-3-mG and its aglucon were determined by TLC in the same conditions as phenolic acids. Composition of the solvent was MeOH:H_2O:HCOOH, 40:50:10 (v:v:v) temperature was higher (20°C). Detection was done at 280 and 520 nm. In these conditions the R_f were: malvidin-3-mG, 0·23; malvidin, 0·12.

Results

Incidence of phenolic acids and anthocyanins on the survival of L. oenos

Survival of *L. oenos* in phosphate buffer, with different phenolic compounds added, was measured. Results obtained after 48 h are reported in Table 1. With a significant variation, gallic acid and free anthocyanins strongly affected cell viability. These two components had a positive effect. Other phenolic acids had no significant influence. However, it should be noted vanillic acid had a slight negative effect. In the following work, only gallic acid, vanillic acid and free anthocyanins were studied.

Interactions between phenolic acids and L. oenos

Influence on growth. Various cultures, control and the medium with added gallic acid or vanillic acid (100 mg l^{-1} of each phenolic acid), were inoculated with 10^8 cell ml^{-1}. Gallic acid significantly affected the growth rate of *L. oenos* (Fig. 1). The growth

Table 1. Effect of phenolic acids and anthocyanins on survival of *L. oenos* in phosphate buffer at pH 4·5

	log N_t/log N_0*	Standard deviation
Control	0·58	±0·03
Gallic acid	0·76	±0·04
Protocatechuic acid	0·59	±0·02
vanillic acid	0·53	±0·02
anthocyanins	0·78	±0·04

*: No, initial viable cell number; N_t, viable cell number after 48 h.
(Concentration: 50 mg l^{-1} for phenolic acids; 100 mg l^{-1} for free anthocyanins; values are the average of three determination).

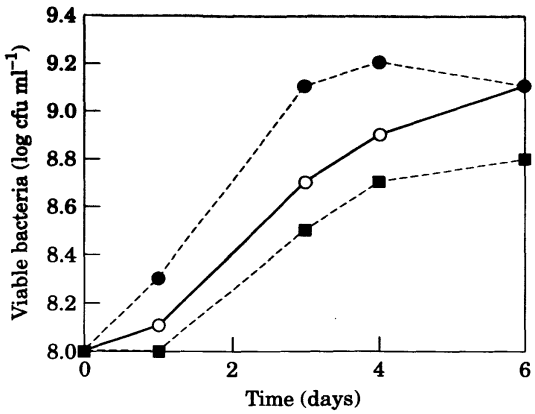

Figure 1. Influence of gallic acid and vanillic acid on the growth of *L. oenos* (Carr medium; temperature, 20°C; s.d.=8%) (○) control; (●) +gallic acid; (■) +vanillic acid.

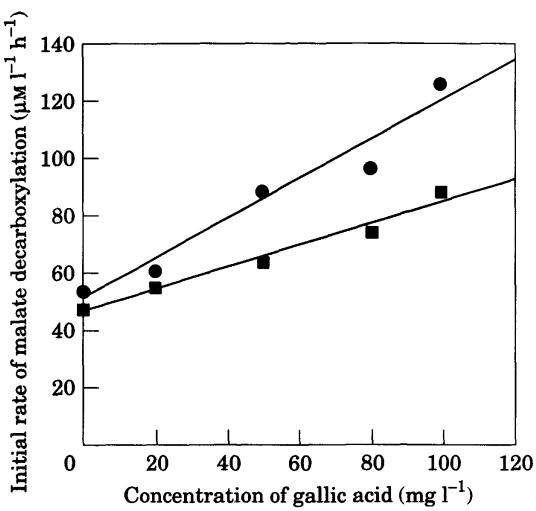

Figure 2. Role of gallic acid on the initial rate of malic acid decarboxylation by *L. oenos* in two different media (temperature, 20°C; s.d.=6%). (●) Carr medium; (■) decolorized wine (charcoal treated).

phase was faster in the gallic acid medium than in the control. In stationary phase the population reached the same cell density as the control; no difference was observed during this phase and subsequent phases. Experiments repeated in quadruplicate gave the same results. Contrary to the results with gallic acid, vanillic acid showed an inhibitory effect on the growth rate and on the optimal population at the stationary phase.

Influence on malic acid degradation. In decolorized wine and in Carr medium, increasing amounts of gallic acid (to 0 at 100 mg l^{-1}) were added, and malic acid decarboxylation was followed (Fig. 2). Gallic acid clearly influenced the decarboxylation rate during malolactic fermentation, both in Carr medium and in decolorized wine. It increased the activity as a linear function of gallic acid concentration. In decolorized wine the correlation constant was r=0·98 at a threshold of 99·9% (P<0·001).

Following these results, the measurement of malolactic activity of resting cells was performed. CO_2 released by decarboxylation of malic acid was measured with the P_{CO_2} electrode. Gallic acid was added to the medium at concentration from 1–100 mg l^{-1}. The decarboxylating rate was influenced by gallic acid. The activity varied from 0·66–1·2 μ Co_2mole mn^{-1} l^{-1} when gallate was added from 0 to 100 mg l^{-1}. Gallate alone was not decarboxylated.

Evolution of phenolic acids. The fate of phenolic acids and their relation to the bacteria metabolism was investigated. First we studied the O.D. at 280 nm during the culture of *L. oenos* with gallic acid and vanillic acid (Table 2). Initially, the O.D. (280 nm) for control medium (1·3) and the two supplemented media (1·8) resulted from the absorbancy of phenolic nucleus. After 12 days the O.D. 280 nm of the gallic acid medium dropped to 1·4. It was the same as for the control and the medium added with vanillic acid. Determination of each phenolic acid in the different media provided more information (Table 3). The assays were conducted using growing cells, non-growing viable cells and heat-inactivated cells.

Table 2. Evolution of O.D. 280 nm during culture of *L. oenos*

Time (days)	Control	Plus gallic acid	Plus vanillic acid
0	1·3	1·8	1·9
5	1·2	1·3	1·8
12	1·2	1·4	1·8

(Control, Carr medium; concentration of phenolic acids, 100 mg l^{-1}).

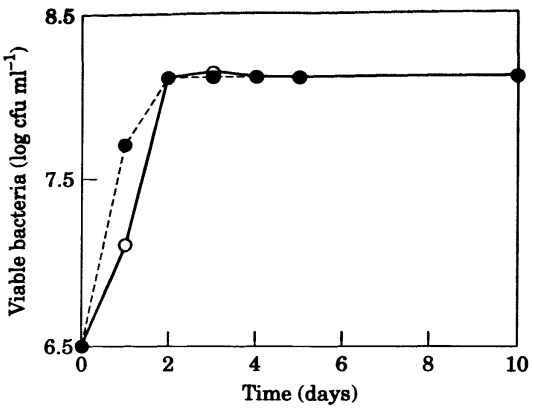

Figure 3. Influence of free anthocyanins on the growth of *L. oenos*. (Carr medium; temperature, 20°C; s.d.=11%). (○) Control; (●) +anthocyanins.

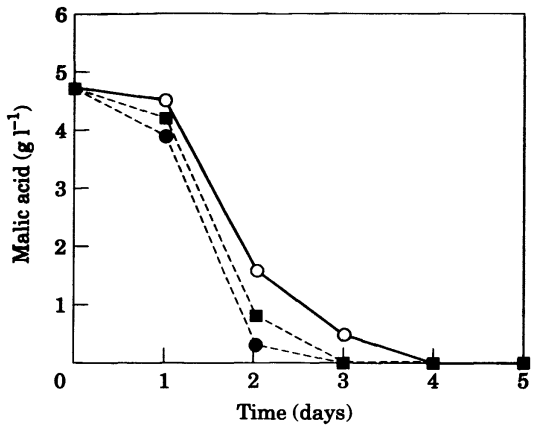

Figure 4. Influence of free anthocyanins and malvidin-3-mG on the range of malolactic fermentation. (Carr medium; temperature, 20°C; concentration of anthocyanins, 200 mg l^{-1}; s.d.=5%). (○) Control; (●) +anthocyanins; (■) +malvidin-3-mG.

Comparison of phenolic content of the various samples at different times showed that only gallic acid disappeared during growth of *L. oenos*. The results after 6 days did not show any significant change when cells were inactivated or non-growing but viable. Moreover, the vanillic acid concentration remained unchanged in all cases.

Interactions of anthocyanins and L. oenos

Influence of anthocyanins on malolactic fermentation. Carr medium was used with 200 mg l^{-1} free anthocyanins powder added. After inoculation by *L. oenos* the population was counted (Fig. 3) and the rate of MLF measured (Fig. 4). Free anthocyanins showed a very limited effect on the growth of *L. oenos* except a slight activating effect during the early growth phase (Fig. 3). Nevertheless, malic acid degradation was stimulated by free anthocyanins and began before the control and had a faster rate of malate degradation (Fig. 4). However, the result could have been attributed to residual phenolic acids contained in free anthocyanins fraction, so the experiments were repeated with pure malvidin-3-mG. The results were approximately the same (Fig. 4): free anthocyanins had a stimulatory effect on malolactic fermentation.

Malvidin was then added to the reaction mixture for determination of malolactic activity by CO_2 measurement. The decarboxylation rate of malic acid was the

Table 3. Influence of viable bacteria cells (growing cells and resting cells) and inactivated cells of *L. oenos* on gallic acid and vanillic acid

Time (days)	Control†		Growing cells*		Inactivated cells†		Resting viable cells†	
	Gallic acid	Vanillic acid	Gallic acid	Vanillic acid	Gallic acid	Vanillic acid	Gallic acid	Vanillic acid
0	100	100	100	100	100	100	100	100
1	100	100	78	100	100	100	100	100
2	100	100	64	97	98	96	92	100
6	82	96	0	95	86	88	85	95

*: Carr medium; †: phosphate buffer.
(Inactivation was conducted by heating at 100°C for 20 min; concentration of phenolic acids, 100 mg l^{-1}; results are percentage of initial quantities of phenolic acids determined by HPTLC.)

same in the control and the assay (0 without malate+200 mg l^{-1} malvidin; 0·66 with malate only; 0·62+50 mg l^{-1} malvidin; 0·66+200 mg l^{-1} malvidin).

Evolution of anthocyanins during bacterial growth. Various media were prepared to follow total free anthocyanins in various conditions: without any lactic acid bacteria, with viable cells of *L. oenos* (growing and non-growing), and with inactivated cells (heated 20 min at 100°C) (Table 4). Free anthocyanins decreased after 5 days by chemical oxidation (–15·5%) in the control. But the decrease was greater in the test case: with inactivated cells (–32·5%), with viable non-growing cells (–54%) and above all with growing cells (–81·5%). This demonstrated that degradation by spontaneous chemical oxidation was not the major mechanism involved, and that adsorption of free anthocyanins on cell walls was limited: viable cells could actually degrade anthocyanins.

Viable bacterial cells were studied in non-growing conditions with 200 mg l^{-1} malvidin-3-mG added to the medium. After 24 h the population was 10^6 cfu ml^{-1} in the control and 10^7 cfu ml^{-1} in the medium with added malvidin-3-mG. At the same time, 20% of malvidin-3-mG disappeared, and after 8 days, no malvidin-3-mG remained. During this experiment, no free malividin was identified. Moreover a precipitate was formed when one volume of medium was added to nine volumes of ethanol. This precipitate was identified as polysaccharide (Dubourdieu 1982, Llauberes-Canal 1989). This polysaccharide absorbed in UV light at 280 nm, whereas usually pure polysaccharides have limited UV absorbance (results not shown). The red color of the ethanol precipitate produced only during the first days of experiments could be partially extracted by isoamyl alcohol; TLC analysis of the extract revealed a component which had the same R_f and the same UV/visible spectrum as malvidin. Soluble fractions, unprecipitable in ethanol, corresponded to polymeric compounds after TLC analysis (Glories 1978). After 4 or 6 days the entrapment in polysaccharide was probably stronger and no colored phenolic compounds could be extracted.

Discussion

Various phenolic compounds, in culture broth and red wines, modified (inhibited and stimulated) bacterial growth and MLF rate. The effect depended on the phenolic compound. In most cases, these compounds were not neutral towards *L. oenos*. A preliminary study in non-growing conditions showed that protocatechuic acid, had no effect on cell viability, inhibation of vanillic acid and activation of gallic acid and free anthocyanins (Vivas et al. 1994, 1995). The mechanisms involved are probably complex. The inhibitory effect cannot be explained by simple adsorption on cell walls as described for fatty acids (Carson and Daneo-Moore 1980, Garbay and Lonvaud-Funel 1990).

Gallic acid activated the early growth phase, but it did not change the optimal population, which was reached at the same time as in the control. It acted as a growth factor (Hajime 1973, Lafon-Lafourcade 1973). Gallic acid also enhanced malate degra-

Table 4. Evolution of total anthocyanins only, with viable *L. oenos* cells (growing and resting), and with inactivated cells

Times (days)	1 Anthocyanins*	2 Plus growing cells†	3 Plus resting cells*	4 Plus inactivated cells*
0	200	200	200	200
1	200	156	171	194
2	182	94	138	180
5	169	37	92	135

*: phosphate buffer; †: Carr medium.
(Results express in mg l^{-1} of total anthocyanins).

dation: the increase was proportional to the quantity of gallic acid added to the medium, and it did not depend on the nature of the medium. MLF lasted 12 days in presence of 100 mg l^{-1} gallic acid and 28 days in the control without gallic acid. These results could be mainly attributed to the higher biomass formed during the first 5 days with gallic acid. Also gallic acid influenced the specific malolactic activity of non-growing cells. In addition, it contributed to the better survival of *L. oenos*, and this may also have enhanced the degradation of malic acid during the stationary phase. Alternatively, growing cells could metabolize gallic acid but autoxidation and adsorption on cell walls were very limited in the experiments. Glucose metabolism was not indispensable, but induced a fast and complete degradation of gallate. The same metabolism is described in *Penicillium* sp. (Maujean et al. 1985). Microbial degradation of different phenolic compounds to flavonoids and phenolic acid by various fungi were reported by Barz (1978). The author demonstrates the ability of micro-organisms to disintegrate flavonoids in phenolic acids in a first step, then disintegrate carboxylic acid to oxaloacetate, acetate, succinate and pyruvate. Bacteria use similar pathways. Phenolic acids are metabolized into organic acids by the β-ketoadipic acid pathway (Evans 1969). In lactobacilli, *L. pastorianus* and *L. plantarum*, Whiting and Cogan (1971) and Whiting (1975) demonstrate that under anaerobic conditions, reduction of quinate or shikimate to dehydroshikimate may replace the reduction of pyruvate to lactate in sugar metabolism. Oxidation of lactate to acetate and CO_2 occurs after sugar exhaustion. *L. plantarum* also has an oxidative pathway, under anaerobiosis, which produces catechol from dehydroshikimic acid. These reactions are, of course, beneficial for the growth of these lactobacilli strains under anaerobiosis. The influence of gallic acid on growth of *L. oenos* might be explained by such a pathway, which needs to be further studied.

Free anthocyanins had a limited effect on the growth of the lactic acid bacteria, and an influence on the rate of malolactic fermentation. The acceleration in MLF was not due to an increase in specific malolactic activity. Anthocyanins had a growth-stimulating effect which resulted in a higher biomass during the first 2 days where more than 90% of malic acid was degraded. The decrease in free anthocyanins in the medium inoculated with inactivated cells was due both to autoxidation and/or adsorption on cell walls. However, a great proportion of anthocyanins, were metabolized by viable lactic acid bacteria, especially when they could grow. Even in non-growing conditions (phosphate buffer) a significant increase in population occured; this suggested that the glucose moeity of the anthocyanins was used as an energy source; this is a very limited factor for increase in bacterial biomass in the reds wine. The polysaccharide, which could be precipitated by ethanol, contained malvidin, as shown by TLC analysis. The anthocyanins could be extracted more easily in the first few days. Therefore, we assumed the production of a stable arrangement polysaccharide–malvidin. Bacteria might have cleaved malvidin-3-mG and used glucose as the carbohydrate source, then malvidin was eliminated by fixation on polysaccharides or peptidoglycan and liberated into the external medium. This is in accordance with previous results (Paraskevopoulos 1988) which established the β-glucosidase activity of *L. oenos*. Moreover hydrolysis of esculin, a glycoside of esculetin, is a very common character of *L. oenos* (Garvie 1986).

Alternatively, the potential role of phenolic compounds as hydrogen acceptors must be considered. Several molecules such as intermediary compounds of metabolism or components of the medium can play this role. Heterofermentative lactic acid bacteria, like *L. oenos*, grow faster when fructose (which is reduced to mannitol) is present (Ribereau-Gayon et al. 1975). This was also noted by Stamer and Stoyla (1970) who suggested that fructose arising from sucrose by autoclaving is responsible for the positive effect on growth of tomato juice; however, it was later shown that a derivative of pantothenic acid is more likely involved (Yoshizumi 1975). In wine, very low amounts of glucose and fructose support the growth of *L. oenos* during MLF. Any molecule acting either as a fermentable substrate or as hydrogen acceptor

has a positive effect on growth. Some phenolic compounds studied in the present work may be involved by releasing even low amounts of fermentable sugar or by reduction. Vetsch and Lüthi (1964) showed that red wine color decreases during malolactic fermentation. They related these results to degradation of citric acid by *Leuconostoc*, linked to reduction of red pigments. However, the reduction of anthocyanins is only possible by strong chemical reductive molecules ($TiCl_3$ for example, unpublished results) or by reducing enzymatic pathways. Quinates, potent hydrogen acceptors, are easily reduced by malolactic bacteria and stimulate growth (Whiting and Coggins 1971). Even if we did not find flavanols i.e. reduced products of anthocyanins in culture media, part of our results sustained this hypothesis.

References

Barz, W. (1978) Microbial degradation of flavonoïds, isoflavonoïds and isoflavonoïd phytoalexins. In *Groupes polyphenols No 8*, (Ed INRA) pp. 63–90. Narbonne.

Carr, J. G. (1956) The occurrence and role of lactic acid bacteria in cider making. *pH. D. Thesis. University of Bristol.*

Carson, D. D. and Daneo-Moore, L. (1980) Effects of fatty acids on lysis of *Streptococcus faecalis*. *J. Bacteriol.* **141,** 1122–1126.

Dubourdieu, D. (1982) Recherches sur les polysaccharides de *Botrytis cinerea* liberés dans les baies de raisins. *Thèse doctorat ès science. Université de Bordeaux II.*

Evans, W. C. (1969) Malolactic fermentation. In *Fermentation advances* (Ed. Perlman, H.) pp. 649–687. Academic Press, London.

Hajime, Y. (1973) Bacteries lactiques et facteurs de croissance. In *Lactic acid bacteria in beverages and food*, Fourth Long Ashton Symposium pp. 243–258. Academic Press, London, New–York, San–Francisco.

Garbay, S. and Lonvaud-Funel, A. (1990) Etude de la lyse de *Leuconostoc eonos*. *J. Int. Vigne Vin* **24,** 157–166.

Garvie, E. I. (1986) Genus *Leuconostoc*. In *Bergey's Manual of Systematic Bacteriology* (ed. Sneath, P. H. A.) pp. 1071–1075. Williams and Wilkins, Baltimore.

Glories, Y. (1976) Recherches sur la structure et les propriétés des composés phénoliques polymérisés des vins rouges. III- Fractionnement des composés phénoliques avec la polyvinylpolypyrrolidone. *Connaissance Vigne Vin* **10,** 51–71.

Glories, Y. (1978) Recherches sur la matière colorante des vins rouges. *Thèse doct. ès sciences, Université de Bordeaux.*

Glories, Y. (1984) La couleur des vins rouges. II- Mesure, origine et interprétation. *Connaissance Vigne Vin* **18,** 253–271.

Kunkee, R. E. (1991) Some roles of malic acid in the malolactic fermentation in wine making. *FEMS Microbiol. Rev.* **88,** 55–71.

Lafon-Lafourcade, S. (1973) Factors of the malolactic fermentation of wines. In *Lactic acid bacteria in beverages and food*, Fourth Long Ashton Symposium pp. 124–132. Academic Press London, New–York, San–Francisco.

Laubberes-Canal, R. M. (1989) Recherches sur les polysaccharides exocellulaires de *Scharomyces cerevisiae* et *Pediococcus* sp. *Thése de l'Université de Bordeaux II.*

Lonvaud, M. (1975) Recherches sur l'enzyme des bactéries lactiques du vin assurant la transformation du malate en lactate. *Thèse 3° cycle, Université de Bordeaux.*

Lonvaud-Funel, A., Joyeux, A. and Desens, C. (1988) The inhibition of malolactic fermentation of wines by products of yeast metabolism. *J. Food Sci. Technol.* **44,** 183–191.

Lonvaud-Funel, A. and Joyeux, A. (1993) Antagonism between lactic acid bacteria of wines: inhibition of *Leuconostoc oenos* by *Lactobacillius plantarum* and *Pediococcus pentosaceus*. *Food Microbiol.* **10,** 411–419.

Lonvaud-Funel, A. (1994) La désacidification biologique des vins. Etat de la question. Perspectives d'avenir. *J. Int. Sci. Vigne Vin* **28,** 161–170.

Maujean, A., Millery, H. et, Lamaresquier, P. (1985) Explications biochimiques et métaboliques de la confusion entre goût de bouchon et goût de moisi. *Rev. Fr. OEnol.* **99,** 55–62.

Paraskevopoulos, Y. (1988) Utilisation des enveloppes cellulaires de levure pour la stimulation de la fermentation malolactique. Interprétation de leur mode d'action. *Thèse docteur-ingénieur, Université de Bordeaux II.*

Peynaud, E. (1967) Etudes récentes sur les bactéries lactiques du vin. *CR II° Symposium international d'oenologie*, Bordeaux. Vol 1, pp. 219–232.

Ribéreau-Gayon, P. and Stonestreet, E. (1966) Le dosage des anthocyanes dans les vins rouges. *Bull. Soc. Chim.* **9,** 2649–2652.

Ribéreau-Gayon, J., Peynaud, E., Ribereau-Gayon, P. et Sudraud, P. (1975) Traité d'oenologie, Tome II, Bordas ed., Paris.

Saraïva, R. (1983) Contribution à l'étude de l'incidence des composés phénolics à l'égard du métabolisme des levures et des bacteries lactiques du vin. Thèse 3° cycle Université de Bordeaux II.

Scalbert, A. (1992) Antimicrobial properties of tannins. *Phytochemistry* **30,** 3875–3883.

Stamer, J. R. and Stoyla, B. O. (1970) Growth

stimulants in plant extracts for *Leuconostoc citrovorum*. *Appl. Microbiol.* 20, 672–676.

Vetsch, V. and Lüthi, H. (1964) Farstoffverlust während des biologischen säureabbauss von rotweinen. *Mitt. Gebiete Lebensm. Hyg.* **55,** 93–98.

Vivas, N., Bellemere, L., Lonvaud-Funel, A., Glories, Y. and Augustin, M. (1994) Etude sur la fermentation malolactique des vins rouges en barriques et en cuves. I° partie. *Rev. Fr. OEnol.* **34,** 37–42.

Vivas, N., Bellemere, L., Lonvaud-Funel, A., Glories, Y. and Augustin, M. (1995) Etude sur la fermentation malolactique des vins rouges en barriques. II° partie. *Rev. Fr. OEnol.* **35,** 39–45.

Whithing, G. G. (1975) Lactic acid bacteria in ciders and alcoholic beverages. In *Lactic acid bacteria in beverages and food* (Eds Carr, E., Cutting, A. and Whithing, G. G.) pp. 254–272. Academic Press, London.

Whithing, G. C. and Coggins, R. A. (1971) The role of quinate and shikimate in the metabolism of lactobacilli. *Antonie van Leeuwenhoek* **37,** 33–49.

Yoshizumi, H. (1975) A malolactic bacterium and its growth factor. In *Lactic acid bacteria in beverages and food* (Eds Carr, E., Cutting, A. and Whithing, G. G.) pp. 254–272. Academic Press, London.