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ScienceDirect
Food Control 19 (2008) 835–841
FOOD CONTROL
www.elsevier.com/locate/foodcont

Review

Potential of phenolic compounds for controlling lactic acid bacteria growth in wine

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Received 22 June 2007; received in revised form 23 August 2007; accepted 28 August 2007

Abstract

Lactic acid bacteria are important in enology since they undergo the malolactic fermentation, a process which main effect is the reduction of wine acidity and is almost indispensable in red wine-making. However, if this process is not well controlled during the elaboration of wine, alterations in wine quality due to bacteria metabolic activity can happen. Polyphenols are wine natural components in must and wine that can potentially affect the growth of lactic acid bacteria and the malolactic fermentation. In this paper, after describing the main features of the malolactic fermentation in wine, we review the use of different chemical substances to control growth of lactic acid bacteria in enology. Special attention is given to phenolic compounds, being revised the recent studies about the effect of polyphenols on the growth and metabolism of lactic acid bacteria in wine in order to establish the extent to which these compounds are involved in malolactic fermentation during wine-making. Finally, the potential use of phenolic extracts as new *antimicrobial* agents during wine-making, as a total or partial alternative to traditional treatments mainly using sulphur dioxide (SO_2) is discussed.

Keywords: Wine; Phenolic compounds; Lactic acid bacteria; Antimicrobial activity; Sulphur dioxide

Contents

1. Introduction

In recent studies, carried out in synthetic laboratory media, the effects of some phenolic compounds (mainly phenolic acids and their esters and some flavonols, such as catechin) on some wine lactic acid bacteria species has

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0956-7135/$ - see front matter
doi:10.1016/j.foodcont.2007.08.018

been studied, revealing that, concentrations of these compounds similar to those found in wine, stimulate bacterial growth (Campos, Couto, & Hogg, 2003; Rozès, Arola, & Bordons, 2003). A possible explanation for the stimulating effects of these compounds, is that they serve as substrate for the bacteria. In fact, research carried out by our group (Hernández et al., 2007) and by other groups (Alberto, Farias, & Manca de Nadra, 2001), has shown that some hydroxycinnamic acids and their esters are metabolized during the growth phase of some lactic acid bacteria species. In contrast, at high concentrations, phenolic compounds are toxic for the bacterial cell, which could cause inhibition of their growth (Reguant, Bordons, Arola, & Rozès, 2000; Stead, 1993). Stimulation or inhibition of the growth of lactic acid bacteria by some wine phenolic compounds, lead us to consider whether they are in any way involved in the development of malolactic fermentation in wine and, also, the possibility of evaluating their use as *natural* antimicrobial agents during wine-making. In this paper, after describing the main features of the malolactic fermentation in wine (Section 2), we review the use of different chemical substances to control growth of lactic acid bacteria (Section 3). Phenolic compounds, that naturally occur in grapes and wines (Section 4), have shown to interact with wine lactic acid bacteria (Section 5), which points out their potential use as new antimicrobial agents in enology (Section 6).

2. Lactic acid bacteria in wine and malolactic fermentation

Together with yeasts, lactic acid bacteria are the most important microorganisms in wine-making. Yeasts are responsible for alcoholic fermentation, while lactic acid bacteria carry out the process of malolactic fermentation (MLF), which, under favorable conditions takes place after alcoholic fermentation. The works carried out in recent years, especially since the eighties, have confirmed the essential role of MLF in wine-making, not only because it reduces the wine acidity, which is very important in red wines, but also because it contributes to the microbial stability of the final product and its organoleptic quality (Maicas, 2001; Moreno-Arribas & Polo, 2005; Versari, Parpinello, & Cattaneo, 1999).

Wine lactic acid bacteria have a complex ecology and, as occurred during the production of many other fermented food products, there is a steady growth of lactic acid bacteria during vinification. Lactic acid bacteria may be present during the different steps of wine-making. They can be isolated from vine leaves, grapes, equipment in the wineries, barrels, etc. The bacteria present in the first steps of wine-making (must and the start of fermentation) belong to different species, generally homofermentative ones. The most abundant correspond to *Lactobacillus plantarum*, *Lb. casei*, *Lb. hilgardii*, *Leuconostoc mesenteroides* and *Pediococcus damnosus*. To a lesser extent, *Oenococcus oeni* and *Lb. brevis* are found. Bacterial multiplication takes place in the interval between the end of alcoholic fermentation and the start of malolactic fermentation. During this step, the pH of the medium, the SO_2 contents, the temperature and the ethanol concentration (Boulton, Singleton, Bisson, & Kunkee, 1996) are the most influential factors. However, conditions specific to each wine, mainly the contents of phenolic compounds can also affect the growth of lactic acid bacteria (Vivas, Augustín, & Lonvaud-Funel, 2000), although this effect is not yet completely understood. *O.oeni* is the bacteria species predominating at the end of alcoholic fermentation. This is the species best adapted to growing in difficult conditions imposed by the medium (low pH and high ethanol concentration) (Davis, Wibowo, Eschenbruch, Lee, & Fleet, 1985; Van Vuuren & Dicks, 1993) and is, therefore, the main species responsible for MLF in most wines. However, some strains of the genera *Pediococcus* and *Lactobacillus* can also survive this phase, remaining active during wine production. If proliferation of these lactic acid bacteria species or strains occurs at the wrong time during wine-making, they may diminish the quality and acceptability of the wine. After MLF, bacterial survival depends on the conditions of the medium, especially on the pH, ethanol contents and, also, particularly on the SO_2 concentration. It is, therefore common practice to remove lactic acid bacteria by sulphiting, after all the malic acid in the wine has been degraded. The levels of sulphurous required to slow down the activity of the lactic acid bacteria oscillate between 10 and 30 mg/l of *free* SO_2 in the case of wines with a pH between 3.2 and 3.6 and from 30 to 50 mg/l for wines with pHs from 3.5 to 3.7. For wines with higher pHs, which is increasingly common in wines from warn areas, the dose of *free* SO_2 required can even reach values close to 100 mg/l.

On some occasions, during industrial wine-making, the development of lactic acid bacteria and MLF are unpredictable, since this can occur during alcoholic fermentation or even during storage or ageing. In these cases, as a consequence of the metabolism of these bacteria, changes occur in the wine composition that can alter its quality, in some cases producing a product which is unacceptable for consumption. These alterations include the so-called "lactic disease", the production of undesirable aromas due to the formation of volatile phenols or aromatic heterocyclic substrates (Chatonet, Dubourdieu, & Boidron, 1995; Costello & Henschke, 2002), and the production of biogenic amines (Landete, Ferrer, Polo, & Pardo, 2005; Marcobal, Polo, Martín-Álvarez, Muñoz, & Moreno-Arribas, 2006; Moreno-Arribas, Torlois, Joyeux, Bertrand, & Lonvaud-Funel, 2000). Biogenic amines are important in wines, not only from a toxicological point of view since they can cause undesirable physiological effects in sensitive humans, such as headache, nausea, hypo-or hypertension, cardiac palpitations, and anaphylactic shock, but also because they could cause problems in wine commercial transactions. Generally, strains identified to cause these problems belong to the group of *Lactobacillus* and *Pediococcus*. Therefore, in wine-making, it is especially important to effectively control MLF, to avoid possible bacterial alterations. On the other hand, although MLF is sometimes difficult to induce in wineries, prevention or inhibition of the growth and development of lactic acid bacteria in wine is also a difficult task.

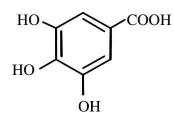
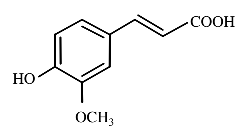
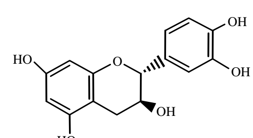
3. The use of SO_2 and complementary substances to control growth of lactic acid bacteria in enology

Sulphur dioxide (SO_2) has numerous properties as a preservative in wines, these include its antioxidant and selective antimicrobial effects, especially against lactic acid bacteria. Today, this is, therefore, considered to be an essential treatment in wine-making. However, the use of this additive is strictly controlled, since high doses can cause organoleptic alterations in the final product (undesirable aromas of the sulphurous gas, or when this is reduced to hydrosuplhate and mercaptanes) and, especially, owing to the risks to human health of consuming this substance. The upper limit permitted by the International Organization of Vine and Wine (OIV) is from 150 to 400 mg/l of total SO_2, depending on the type of wine and its content of reducing matter. However, according to European Union regulations (Ruling n°1622/2000), the total SO_2 content in red wines cannot exceed 160 mg/l, and in white wines it cannot exceed 210 mg/l. On the other hand, in the United States, and also recently in the European Union (specifically from the 26 November 2005, Ruling n° 1991/2004), the legislation requires wine-makers, to specify the presence of sulphites on the wine label, in cases where these exceed 10 mg/l. In fact, in most wines, it is increasingly common to find the specification "*contains sulphites*" on a visible part of the label.

Because of these effects, in recent years there is a growing tendency to reduce the maximum limits permitted in musts and wines. Although as yet, there is no known compound that can replace SO_2 with all its enological properties, there is great interest in the search for other preservatives, harmless to health, that can replace or at least complement the action of SO_2, making it possible to reduce its levels in wines.

With regards products with antimicrobial activity complementary to SO_2 (Table 1), recently dimethyldicarbonate (DMDC) has been described as being able to inhibit alcoholic fermentation and development of yeasts, permitting the dose of SO_2 to be reduced in some types of wines (Divol, Strehaiano, & Lonvaud-Funel, 2005; Threlfall & Morris, 2002). Yeast cells have been shown to die after adding this compound, whereas with SO_2 they enter a "viable state but cannot be cultivated" (Divol et al., 2005), which has also been demonstrated for lactic acid bacteria (Millet & Lonvaud-Funel, 2000). Other alternatives have been introduced based on "natural antimicrobial agents", of which the use of lysozyme is especially important (Bartowsky, 2003; Gerbaux, Villa, Monamy, & Bertrand, 1997), and some antimicrobial peptides or bacteriocins (Du Toit, du Toit, Krieling, & Pretorius, 2002; Navarro, Zarazaga, Sáenz, Ruiz-Larrea, & Torres, 2002) (Table 1).

Table 1
Other compounds proposed to control lactic acid bacteria growth in enology

Compound	Chemical characteristics	References
Dimethyldicarbonate (DMDC)	$(CH_3OCO)_2O$	Threlfall and Morris (2002), Divol et al. (2005)
Lysozyme	Enzyme obtained from egg white (129 amino acids)	Gerbaux et al. (1997), Bartowsky (2003)
Bacteriocins	Nisin (pM < 5000; 34 amino acids) Pediocin PD-1 (pM 2866 pI 9,0; optimum pH 5.0 at 25 °C)	Radler (1990), Rojo-Bezares et al. (2007) Bauer et al. (2003), Bauer et al. (2005)
Polyphenols	Gallic acid  Ferulic acid  (+)-Catechin 	Vivas et al. (1997), Reguant et al. (2000)

In the case of lysozyme, since this was first authorized as an additive in wine-making it has only been used very little due to the high costs of its application. Another aspect to take into account about this protein is that it can cause IgE-mediated (Mine & Zhang, 2002) immune reactions in some individuals so its presence in food products, including wine, can cause some concern. To date, nisin is the only bacteriocin that can be obtained commercially, and although this has been shown to be effective at inhibiting the growth of spoilage bacteria in wines (Radler, 1990; Rojo-Bezares, Sáez, Zarazaga, Torres, & Ruiz-Larrea, 2007), it has not been authorized for use in enology. Other bacteriocins have been described to control the growth of lactic acid bacteria in wine, although the efficacy of these compounds, their mode of action and, especially, their stability during wine-making are still under investigation (Bauer, Hannes, & Dicks, 2003, 2005) (Table 1).

4. Wine phenolic compounds

Phenolic compounds or polyphenols are natural constituents of grapes and wines. Under the name of polyphenols, numerous compounds of different chemical structure are grouped together including: hydroxybenzoic acids, hydroxycinnamic acids, stilbenes, alcohols, flavanols, flavonols, anthocyanins and tannins. These compounds are very important since they are responsible for many of the organoleptic properties of wines, especially, color and astringency. Wine polyphenols are also associated with the beneficial effects associated with moderate wine consumption, especially in relation to cardiovascular diseases. In any case, the structure of a phenolic compound determines its chemical reactivity and its biological properties.

The concentration of phenolic compounds in wine is conditioned by several factors related to the grape (variety, quality of the harvest, soil, climate, etc.) and by enological practices. During wine-making, factors such as maceration time and temperature, fermentation in contact with skins and seeds, the addition of enzymes, the concentration SO_2, the pressing, etc. all affect extraction of phenolic compounds from the grape to the must/wine (Sacchi, Visón, & Adams, 2005). MLF also affects the phenolic composition of wine, reducing the contents of anthocyanins and total polyphenols (Vrhovsek, Vanzo, & Nemanic, 2002). During ageing in the bottle, wine anthocyanin content drops, although the total polyphenol content is less variable (Monagas, Bartolomé, & Gómez-Cordovés, 2005b, 2005a). As a result, the total polyphenol content is around 150–400 mg/l for white wines and 900–1400 mg/l for young red wines.

As a summary, Table 2 shows the whole range of concentrations of the main phenolic compounds identified in young red wines. According to groups of compounds, acids and hydrobenzoic derivatives represent 6% of the total, acids and hydroxycinnamic derivatives 1.1%, stilbenes 0.5%; alcohols 3.8%; flavanols, 15%; flavonols, 3.6%; and anthocyanins, 70%. Other anthocyanin derivatives such as pyranoanthocyanins are present in much lower proportions.

5. Interactions between phenolic compounds and wine lactic acid bacteria

Most studies to date about the interactions between phenolic compounds and lactic acid bacteria in wines refer to the metabolism of hydroxycinnamic acids (ferulic and coumaric acids), by different bacteria species, resulting in the formation of volatile phenols (4-ethylguaiacol and 4-ethylphenol) (Barthelmebs, Diviès, & Cavin, 2001; Cavin, Andioc, Etievant, & Diviès, 1993; Gury, Barthelmebs, Tran, Diviès, & Cavin, 2004). The metabolism of other phenolic compounds such as gallic acid and catechin have also been studied (Alberto, Gómez-Cordovés, & Manca de Nadra, 2004; Vaquero, Marcobal, & Muñoz, 2004). More recently, it has also been reported that *trans*-cafftaric and *trans*-coutaric acids are substrates of wine lactic acid bacteria, that can exhibit cinnamoyl esterase activities during MLF, increasing the concentration of the hydroxycinnamic acids (Hernández et al., 2007, Hernández, Estrella, Carlavilla, Martín-Álvarez, & Moreno-Arribas, 2006).

However, little is known about the effect of wine phenolic compounds on the growth and metabolism of microorganisms, in general, and especially on the lactic acid bacteria that participate in the wine-making process. It has been suggested that phenolic compounds can behave as activators or inhibitors of bacterial growth depending on their chemical structure (substitutions in the phenolic ring) and concentration (Reguant et al., 2000; Vivas, Lonvaud-Funel, & Glories, 1997). For example, it has been demonstrated in *Lb. hilgardii* in culture media that gallic acid and catechin in concentrations found in wines, not only stimulate growth but also increase the bacterial population, owing to their ability to metabolize these compounds during the growth phase, bringing energy to the cell (Alberto et al., 2001). It also seems that they can affect the bacteria metabolism (Rozès et al., 2003; Vivas et al., 2000), since they favor the use of sugars and malic acid (Alberto et al., 2001). On the other hand, at higher concen-

Table 2
Main phenolic compounds identified in young red wines (De Villiers et al., 2005; Monagas et al., 2005a; Monagas et al., 2005b; Soleas et al., 1997)

	Concentration (mg/l)		Concentration (mg/l)
Hydroxibenzoic acids		Flavanols	
Gallic acid	10–37	(+)-Catechin	16–58
Protocatechuic acid	1.2–4.7	(−)-Epicatechin	10–38
Syringic acid	4.2–5.8	Procyanidins B1, B2, B3, B4	14–33
Hydroxycinnamic acids		Flavonols	
Cafftaric acid	0.7–46	Myricetin-3-glycosides	1.6–22
Coutaric acid	0.7–11	Quercetin-3-glycosides	1.3–34
Caffeic acid	0.3–33	Myricetin	1.7–8
p-Coumaric acid	0.1–8	Quercetin	1.9–15
Stilbenes		Anthocyanins	
trans-Resveratrol	0.4–2.5	Delfinidin-3-glucoside	7–11
trans-Resveratrol-3-O-glucoside	0.1–3	Petunidin-3-glucoside	14–25
Alcohols		Malvidin-3-glucoside	170–260
Tyrosol	7–26	Malvidin-3-(6-acetyl)-glucoside	23–108
Tryptophol	nd-4.5	Malvidin-3-(6-caffeoil)-glucoside	3.5–5.6
		Malvidin-3-(6-*p*-coumaroyl)-glucoside	16–28

trations, these compounds have a negative effect on bacterial development. *O. oeni* seems to be more sensitive to inactivation by phenolic compounds than *Lb. hilgardii* (Campos et al., 2003).

Free hydroxycinnamic acids also appear to affect the growth of *Lb. plantarum* and some spoiling species of the group of *Lactobacillus.* Ferulic acid seems to be more effective than *p*-coumaric acid, although some species are more susceptible than others. In contrast, the esters of this acid, as well as the non-phenolic acid, quinnic acid, do not affect growth of *Lb. plantarum* (Salih, Le Quéré, & Drilleau, 2000). Moreover, it has been found that, in a synthetic laboratory environment, the concentration of these compounds can have a critical effect, since the bacteria can tolerate and also metabolize concentrations between 100 and 250 mg/l, which could possibly explain the beneficial effect of these compounds on growth. In contrast, concentrations above 500 mg/l, produce a toxic effect (Stead, 1993). The mechanism of this inhibition is not clear. From these works carried out with pathogenic bacteria, some authors propose that these compounds can act on proteins of the bacteria cell membrane causing a series of compounds to leave the cell interior, producing losses in K^+, glutamic acid, intracellular RNA, etc. as well as an alteration in the composition of fatty acids (Rozès & Perez, 1998). Other authors have suggested that phenols adsorb to the cell walls and alter the cell casing, and even other mechanisms that involve interactions with cellular enzymes (Campos et al., 2003). Recently, a contribution towards the elucidation of the mechanisms of tannins on bacteria growth inhibition was investigated by a combination of physiologic and proteomic approaches (Bossi et al., 2007). The effects of tannic acid on cells are deduced by the involvement of metabolic enzymes, and functional proteins on the tannin–protein interaction.

6. Antimicrobial properties of phenolic compounds

The increased resistance of isolated human and animal pathogens, combined with consumers' growing concern about the use of chemical products as preservatives, has led, over the past few years, to studies being conducted into the application of new efficient antimicrobial products with harmful effects to health. Hence, in recent years, it has gained interest in the study of the antimicrobial properties of phenolic extracts obtained from plants (Ezouberi et al., 2005; Rauha et al., 2000; Zhu, Zhang, & Lo, 2004) and fruits (Puupponen-Pimia, Nohymek, & Hartmann-Schmidin, 2005, 2001). Some studies have been reported in the literature which demonstrate, in growth media, the antimicrobial activity of different phenolic extracts obtained from enological products such as grape seeds (Papadopoulu, Soulti, & Roussis, 2005) and white and red wine (Baydar, Ozkan, & Sagdic, 2004; Rodríguez-Vaquero, Alberto, & MancadeNadra, 2007) against pathogenic bacteria. Phenolic extracts mainly containing phenolic acids, have been described to be more active against bacteria than against yeasts, suggesting that yeasts have a stronger resistance to the action of these compounds. Some attempts have even been made to obtain phenolic fractions, from seeds, with a broad spectrum of activity against bacteria, by "clean" technologies, such as extraction with super-critical fluids, which could constitute a first step for their subsequent development and application in industry (Palma, Taylor, Varela, Cutler, & Cutler, 1999).

As mentioned previously, the efficacy of phenolic compounds as antimicrobial agents against lactic acid bacteria in wine depends on the compound's structure, and is dose-dependent. In general, the antimicrobial effect appears to occur at higher doses than those usually found in wines. Therefore, we must consider that the application of phenolic extracts as antimicrobial agents in wines would be conditioned by possible changes that effective concentrations of these compounds would produce in the physico-chemical (solubility) and organoleptic properties (color, aroma) of the wine. However, it is important to take into account that studies carried out to date (reported above) have been conducted in growth media, in which bacterial growth is favored by the composition and pH of the media. Therefore, the concentration of phenolic compounds required to inhibit growth would be lower in an adverse medium, such as wine (Stead, 1993). On the other hand, antimicrobial activity of phenolic compounds could increase because of synergic effects between them or with other antimicrobial agents, such as SO_2, allowing to reduce the dose of each of them. Finally, when studying the effect of a given phenolic compound, it is important to take into consideration the presence in the wine of other compounds, such as proteins, sugars or oxidants, that can interact with the compound studied, affecting its activity. In any case, studies taking all these factors into consideration are required for establishing the possible applications of phenolics as antimicrobial agents in wine-making.

Acknowledgements

Work in the laboratory of the authors was funded by the Spanish Ministry for Science and Education (AGL2006-04514 and PETRI95-0759 OP Projects), and the Comunidad de Madrid (S-0505/AGR/0153 Project). AGR is the recipient of a fellowship from the CSIC-I3P.

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