Food Control 28 (2012) 212–219

Contents lists available at SciVerse ScienceDirect

Food Control

journal homepage: www.elsevier.com/locate/foodcont

Antimicrobial phenolic extracts able to inhibit lactic acid bacteria growth and wine malolactic fermentation

Almudena García-Ruiz [a], Carolina Cueva [a], Eva M. González-Rompinelli [a], María Yuste [b], Mireia Torres [b], Pedro J. Martín-Álvarez [a], Begoña Bartolomé [a], M. Victoria Moreno-Arribas [a,*]

[a] Instituto de Investigación en Ciencias de la Alimentación (CIAL), CSIC-UAM, C/ Nicolás Cabrera 9, Campus de Cantoblanco, Universidad Autónoma de Madrid, 28049 Madrid, Spain
[b] Bodegas Miguel Torres S.A.M. Torres 6, 08720 Vilafranca del Penedés, Barcelona, Spain

ARTICLE INFO

Article history:
Received 12 January 2012
Received in revised form
23 April 2012
Accepted 1 May 2012

Keywords:
Wine
Phenolic extracts
Lactic acid bacteria
Acetic acid bacteria
Malolactic fermentation
Antimicrobial activity
Alternatives to SO_2

ABSTRACT

The purpose of this study was to determine whether phenolic extracts with antimicrobial activity may be considered as an alternative to the use of sulfur dioxide (SO_2) for controlling malolactic fermentation (MLF) in winemaking. Inhibition of the growth of six enological strains (*Lactobacillus hilgardii* CIAL-49, *Lactobacillus casei* CIAL-52, *Lactobacillus plantarum* CIAL-92, *Pediococcus pentosaceus* CIAL-85, *Oenococcus oeni* CIAL-91 and *O. oeni* CIAL-96) by phenolic extracts ($n = 54$) from different origins (spices, flowers, leaves, fruits, legumes, seeds, skins, agricultural by-products and others) was evaluated, being the survival parameter IC_{50} calculated. A total of 24 extracts were found to significantly inhibit the growth of at least two of the LAB strains studied. Some of these extracts were also active against two acetic acid bacteria (*Acetobacter aceti* CIAL-106 and *Gluconobacter oxydans* CIAL-107). Transmission electron microscopy of the bacteria cells after incubation with the phenolic extract confirmed damage of the integrity of the cell membrane. Finally, to test the technological applicability of the extracts, the eucalyptus extract was added (2 g/L) to an industrially elaborated red wine, and the progress of the MLF was evaluated by means of residual content of malic acid. Addition of the extract significantly delayed the progress of both inoculated and spontaneous MLF, in comparison to the control wine (no antimicrobial agent added), although not as effective as $K_2S_2O_5$ (30 mg/L). These results demonstrated the potential applicability of phenolic extracts as antimicrobial agents in winemaking.

© 2012 Elsevier Ltd. All rights reserved.

1. Introduction

In wines, lactic acid bacteria (LAB) carry out the process of malolactic fermentation (MLF), which takes place after alcoholic fermentation under favorable conditions. Wine deacidification is the main trigger for MLF, and consists of the conversion of L-malic acid to L-lactic acid resulting in a decrease in titratable acidity and a small increase in pH. MLF also contributes to wine microbial stability and improves the complexity of wine aroma (Maicas, 2001; Miller, Franz, Cho, & Du Toit, 2011; Moreno-Arribas & Polo, 2005; Versari, Parpinello, & Cattaneo, 1999).

The bacteria present in the first steps of winemaking (must and the start of fermentation) belong to different species, generally homofermentative ones. The most abundant belong to the species *Lactobacillus plantarum*, *Lactobacillus hilgardii*, *Leuconostoc mesenteroides* and *Pediococcus* sp., while to a lesser extent, *Oenococcus oeni* and *Lactobacillus brevis* are also found. Bacterial multiplication takes place in the interval between the end of alcoholic fermentation and the start of MLF. During this stage, the pH of the medium, the SO_2 content, the temperature and the ethanol concentration (Boulton, Singleton, Bisson, & Kunkee, 1996) are the most influential factors. *O. oeni* is the bacteria species predominating at the end of alcoholic fermentation. This is the species best adapted to growing in the difficult conditions imposed by the medium (low pH and high ethanol concentration) (Davis, Silveira, & 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, and most of them are considered to be wine spoilage species. Consequently, if MLF is not well controlled, alterations in wine quality due to bacteria metabolic activity can happen. It is, therefore, common practice to remove LAB by sulphiting the wine once malic acid has been mostly degraded.

Sulfurous anhydride or sulfur dioxide (SO_2) has numerous properties as a preservative in winemaking; these include its

* Corresponding author.
E-mail address: victoria.moreno@csic.es (M.V. Moreno-Arribas).

0956-7135/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodcont.2012.05.002

antioxidant and selective antimicrobial effects, especially against LAB. Nevertheless, and due to increasing health concerns, consumer preference, possible organoleptic alterations in the final product and a tighter legislation regarding preservatives, there is a worldwide trend to reduce SO_2 levels in wine (du Toit & Pretorius, 2000), with a particular interest within the scientific community in the development of total or partial alternatives to the traditional use of SO_2 in winemaking (Bartowsky, 2009; Fredericks, du Toit, & Krügel, 2011; García-Ruiz et al., 2008; Izquierdo-Cañas, García-Romero, Huertas-Nebreda, & Gómez-Alonso, 2012).

Over the last two decades, other preservatives from plant, animal and microbial origins have been intensely investigated for practical applications (for a review see Pozo-Bayón, Monagas, Bartolomé, & Moreno-Arribas, 2012). In particular, 'natural' products such as polyphenols have been reported to have a variety of biological effects, including antioxidant, anticarcinogenic, anti-inflammatory and antimicrobial activities (Xia, Deng, Guo, & Li, 2010). Phenolic extracts from different vegetal origins, such as rosemary, cocoa, olive oil (Bubonja-Sonje, Giacometti, & Abram, 2011), cranberry (Côté et al., 2011), blueberry (Park, Biswas, Phillips, & Chen, 2011), onion, garlic (Benkeblia, 2004), mango (Kaur et al., 2010), plant and agricultural by-products (Balasundram, Sundram, & Samman, 2006), grape pomace (Özkan, Sagdiç, Baydar, & Kurumahmutoglu, 2004), grape (Baydar, Özkan, & Sagdiç, 2004, Baydar, Sagdiç, Özkan, & Cetin, 2006) and almond skins (Mandalari et al., 2010), have demonstrated their antimicrobial capacity against numerous spoilage and pathogenic bacteria. Most of these references were in pure culture experiments. Other studies carried out on salad vegetables (Karapinar & Sengun, 2007) and meat products such as fresh pork patties (Park & Chin, 2010), beef meatballs (Fernández-López, Zhi, Aleson-Carbonell, Pérez-Alvarez, & Kuri, 2005) and chicken products (Kanatt, Chander, & Sharma, 2010) have demonstrated the potential application of phenolic extracts as antimicrobial and antioxidant agents in order to prevent food diseases and to prolong the shelf life of final products.

With regard to the potential application of polyphenols as preservatives in wines, most studies have evaluated the effects of pure compounds on isolated bacteria (for a review see García-Ruiz et al., 2008). Recently, the inhibitory effects of the different classes of phenolic compounds present in wine (hydroxybenzoic acids and their derivatives, hydroxycinnamic acids, phenolic alcohols and other related compounds, stilbenes, flavan-3-ols and flavonols) on different LAB wine isolates have been compared (García-Ruiz, Bartolomé, Cueva, Martín-Álvarez, & Moreno-Arribas, 2009; García-Ruiz, Moreno-Arribas, Martín-Álvarez, & Bartolomé, 2011), confirming the potential of phenolic compounds as preservatives in winemaking. However, until now, the effectiveness of plant phenolic extracts – which are the products potentially applicable in winemaking – in controlling LAB growth during wine MLF has not been investigated.

With the ultimate goal of developing new alternatives to the use of sulphites in enology, the objective of this work was to evaluate the potential of plant phenolic extracts to inhibit the growth of LAB and the progress of MLF in wines. In the first part of the work, we measured the inhibitory potency of 54 commercial phenolic extracts from different origins on the growth of different enological strains of LAB and acetic acid bacteria (AAB). Results are expressed as IC_{50} in order to allow further comparison between polyphenol structures and bacteria species and strains. In the second part, the efficacy of one of the most active extracts in pure cultures (the eucalyptus extract) was also tested in wine MLF, occurring either spontaneously or by inoculation with a malolactic starter.

2. Materials and methods

2.1. Phenolic extracts

A total of 54 phenolic extracts were assayed: *spices (n = 5)*: cinnamon, eucalyptus, oregano, rosemary and thyme; *flowers (n = 2)*: camomile and yarrow; *leaves (n = 15)*: green tea (n = 3), rock tea, red tea, elder leaves, olive tree leaves, Olixxol® (a commercial formulation from the olive tree), walnut leaves, currant leaves, *Ginkgo biloba,* lady's mantle leaves and vine leaves (n = 3); *fruits (n = 8)*: acerola, apple, bitter orange, bilberry, citrus, Citrolive® (a commercial formulation from the citrus tree) and pomegranate (n = 2); *legumes (n = 2)*: soy bean and red clover; *seeds (n = 4)*: green coffee and grape seeds (n = 3); *skins (n = 6)*: almond skins, Amanda® (a commercial formulation from almond skins) and red grape skins (n = 4); *agricultural by-products (n = 3)*: grape pomace (n = 2), and Eminol® (a formulation from grape pomace); *wine (n = 1)*: Provinols™ (a formulation from red wine); *purified tannins (n = 7)*: grape seed tannins, grape skin tannins, oak tannins, quebracho tannins, Vitaflavan® (a formulation from grape seed tannins) and monomeric and oligomeric fractions from Vitaflavan®; *others (n = 1)*: propolis (Table 1). All phenolic extracts were kindly provided by their producers: Biosearch Life S. A. (Granada, Spain), Agrovin S.L. (Ciudad Real, Spain) and SilvaTeam (San Michele Mondovì, Italy), except the seed and grape skin tannins which were kindly provided by Dr. Vivas (University of Bordeaux 1, France). In general, the extracts were obtained after maceration of the plant material with aqueous alcoholic mixtures at a temperature between 25 and 75 °C, following by a drying process to get a final stable solid powder.

2.2. Determination of total phenolic content and antioxidant activity of the extracts

Phenolic extracts (0.05 g) were mixed with 10 mL of methanol/HCl (1000/1, v/v) and sonicated for 5 min followed by a 15 min resting period. The mixture was then centrifuged (3024 *g*, 5 min, 5 °C) and filtered (0.45 μm) to determine the total phenolic content (total polyphenols, TP). The method of Singleton and Rossi (1965), based on the oxidation of the hydroxyl groups of phenols in basic media by the Folin–Ciocalteu reagent, was used for determining the total phenolic content of the extracts. The results were expressed as mg of gallic acid equivalents per gram of extract. The analysis was performed in triplicate.

For characterization purposes, the radical scavenging activity of the phenolic extracts was determined by the ORAC (Oxygen-Radical Absorbance Capacity) method using fluorescein as a fluorescence probe (Dávalos, Gómez-Cordovés, & Bartolomé, 2004). Briefly, the reaction was carried out at 37 °C in 75 mM phosphate buffer (pH 7.4) and the final assay mixture (200 μL) contained fluorescein (70 nM), 2,2′-azobis(2-methyl-propionamidine)-dihydrochloride (12 mM) and antioxidant (Trolox [1–8 μM] or phenolic extract [at different concentrations]). ORAC values were expressed as mmol of Trolox equivalents per g of extract. The analysis was performed in triplicate.

Correlation analysis (Pearson's correlation coefficient) was used to investigate the relationship between TP and ORAC parameters, using the STATISTICA program for Windows, version 7.1 (StatSoft. Inc. 1984–2006, www.statsoft.com).

2.3. Culture media and growth conditions

Six strains of LAB, *L. hilgardii* CIAL-49, *Lactobacillus casei* CIAL-52, *L. plantarum* CIAL-92, *Pediococcus pentosaceus* CIAL-85, *O. oeni* CIAL-91 and *O. oeni* CIAL-96, and two strains of acetic acid bacteria (AAB)

Download English Version:

https://daneshyari.com/en/article/6394289

Download Persian Version:

https://daneshyari.com/article/6394289

Daneshyari.com