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Antimicrobial and antioxidant activity of pressurized liquid extracts from oenological woods

M.E. Alañón [a, *], A. García-Ruíz [b], M.C. Díaz-Maroto [c], M.S. Pérez-Coello [c], M.V. Moreno-Arribas [b]

[a] Food and Nutritional Sciences Department, School of Chemistry, Food and Pharmacy, University of Reading, Whiteknights, RG6 6AP, Reading, United Kingdom
[b] Instituto de Investigación en Ciencias de la Alimentación (CIAL), CSIC-UAM, C/ Nicolas Cabrera 9, Campus de Cantoblanco, Universidad Autónoma de Madrid, 28049 Madrid, Spain
[c] Área de Tecnología de los Alimentos, Facultad de Ciencias Químicas, Universidad de Castilla-La Mancha, Avd. Camilo José Cela 10, 13071, Ciudad Real, Spain

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ABSTRACT

The main goal of this study was to evaluate the antimicrobial activity of a collection of oenological woods extracts (non-toasted and toasted American oak wood, non-toasted and toasted French oak wood, non-toasted and toasted Rumanian oak wood, chestnut, cherry and wine grape wood) isolated by pressurized liquid extraction in order to control the microbial spoilage of wines. Inhibition of the growth of six wine lactic acid bacteria (LAB) (*Lactobacillus hilgardii* CIAL-49, *Lactobacillus casei* CIAL-52, *Lactobacillus plantarum* CIAL-92, *Pediococcus pentosaceus* CIAL-85, *Oenococcus oeni* CIAL-91 and CIAL-96), two acetic acid bacteria (AAB) (*Acetobacter aceti* CIAL-106 and *Gluconobacter oxydans* CIAL-107) and three *Brettanomyces* yeast (*Brettanomyces bruxellensis* CIAL-108, CIAL-109 and CIAL-110) by the oenological wood extracts was assessed. The antioxidant activity and the total phenol index of wood extracts were also evaluated. Results confirmed differences in bacteria and yeast susceptibility to oenological wood extracts among different genera and species. Among them, AAB were especially sensitive to the phenolic inactivation from oenological woods extracts. Contrarily, amongst LAB, *L. hilgardii* CIAL-49 was the most resistant strain to the action of the wood extracts. Cherry wood was active against 9 of the 11 strains tested meanwhile French, Rumanian oak wood and chestnut show the lowest values of IC_{50} for *A. aceti* CIAL-106. No significant correlation was found between antimicrobial activity either with antioxidant activity or with the total phenol content, suggesting that structure-function of the phenolic extracts has a greater influence on the antimicrobial activity than the total phenol content.

1. Introduction

Wines are characterized by high ethanol content, low acidity and limited nutrients. These environmental conditions can only be supported by a limited number of micro-organisms such as several species of yeast, lactic acid bacteria (LAB) and acetic acid bacteria (AAB). The occurrence of some micro-organisms is desirable to obtain a wine of great quality. However the presence of others and the proliferation of certain species or strains at inappropriate time during winemaking process may diminish the quality and acceptability of the wine.

LAB are responsible for carrying out the process of malolactic fermentation (MLF), an essential step especially in red wines, which takes place after alcoholic fermentation. The development of the MLF presents several benefits in wines such as the reduction of the acidity, the stability of the final product and the enhancement of the organoleptic quality (Maicas, 2001; Miller, Franz, Cho, & Du Toit, 2011; Moreno-Arribas & Polo, 2005; Versari, Paripinello, & Cattaneo, 1999). However, the development of LAB can cause numerous unwelcome wine spoilage problems if it occurs at inappropriate stage of the winemaking process, producing on the one hand undesirable flavours due to the formation of diacetyl, acetic acid and other volatile fatty acids mainly or volatile phenols or aromatic heterocyclic substrates to lesser extent (Chatonnet, Dubourdieu, & Boidron, 1995; Costello & Henschke, 2002) and the other hand, producing biogenic amines which are

* Corresponding author. Tel.: +44 (0) 1183787713; fax: +44 (0) 1189310080.
E-mail addresses: a.p.elena@reading.ac.uk, elenaalanoncr@hotmail.com (M.E. Alañón).

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demonstrated to be toxic due to their undesirable physiological effects in sensitive humans (Landete, Ferrer, Polo, & Pardo, 2005; Marcobal, Polo, Martín-Álvarez, Muñoz, & Moreno-Arribas, 2006; Moreno-Arribas, Torlois, Joyeux, Bertrand, & Lonvaud-Funel, 2000). Therefore it is especially significant to effectively control malolactic fermentation and bacteria spoilage.

Bacterial wine spoilage can also be induced by AAB. The metabolites produced by these bacteria are acetic acid, acetaldehyde and ethyl acetate which have an undesirable impact on the wine sensory qualities (Bartowsky, 2009). When these microorganisms grow in an uncontrolled manner, spoilage of wine occurs and this spoilage can happen at multiple stages of the vinification.

The growth of the spoilage yeast *Brettanomyces/Dekkera* during the winemaking process can cause sensory deterioration of wine due the formation of ethylphenols, 4-ethylphenols and 4-ethylguaiacol, whose sensorial attributes are describe as "phenols", "animal" and "stable" off-odours (Chatonnet, Dubourdieu, Boidron, & Pons, 1992). Although LAB also produce ethylphenols in small quantities, *Brettanomyces bruxellensis* is considered the primary yeast species involved in wine spoilage (Chatonnet et al., 1995).

In order to control and prevent microbial wine spoilage, sulphurous anhydride or sulphur dioxide (SO_2) has been used traditionally as antimicrobial and antioxidant agent in the winemaking process. However, this chemical preservative must be strictly controlled, not only due to its possible organoleptic alterations in the final product, but also due to the risks to human health. For that reasons, there is a tendency of seeking natural alternatives to remove total or partial the use of synthetic preservatives (Pozo-Bayón, Monagas, Bártolome, & Moreno-Arribas, 2012; Señoráns, Ibañéz, & Cifuentes, 2003).

In the last decades, the scientific interest has been focused on antimicrobial peptides or bacteriocins (Díez et al., 2012) and, in particular, on polyphenols from natural sources with an eye towards their possible antimicrobial activity (García-Ruíz et al., 2008; Santos, Nunes, Saraiva, & Coimbra, 2012). However, the effect of the polyphenols on the bacterial growth is not yet well understood and may vary according to the micro-organism and the type, structure, and concentration of polyphenols (large doses of phenolic compounds may be toxic for bacteria but at lower doses they can be used as substrates) (Campos, Couto, & Hogg, 2003; Figueiredo, Campos, de Freita, Hogg, & Couto, 2008; García-Ruíz et al., 2012). Some authors propose that these compounds can interact with the proteins of the bacteria cell membrane causing damage to the integrity of the cell membrane and the subsequent release of the cytoplasm, or can be involved in interaction with cellular enzymes (Campos et al., 2003; García-Ruíz, Bartólome, Cueva, Martín-Álvarez, & Moreno-Arribas, 2009; García-Ruíz et al., 2012; Rozès & Pérez, 1998). On the other hand, phenolic compounds are known to serve oxygen scavenging and reduce the redox potential of wines (Vivas & Glories, 1995; Vivas, Lonvaud-Funel, & Glories, 1997). This property has been tentatively suggested to be related to the effect of phenolic compounds on the growth and metabolism certain bacteria (Reguant, Bordons, Arola, & Rozès, 2000; Theobald, Pfeiffer, Zuber, & König, 2008).

One of the most important practices in the production of some alcoholic beverages is the wood ageing process which makes the finished product highly valued due to the enhancement on the complexity aroma, the extraction of wood phenolic compounds and the microoxygenation through the wood pores. But, it has also proven wood to be the beneficial material in the maturation of wines in barrels in order to control of oxidation and to prevent the wine from excess microbial contamination (Scalbert, 1991). Indeed, wood tannins have been demonstrated to possess antimicrobial properties against pathogenic bacteria (Andrensek et al., 2004; Scalbert, 1991).

Therefore, taking into account that wood used in cooperages is especially rich in phenolic compounds, the aim of this study was to investigate the inactivation properties of different oenological wood extracts as preservative to control or avoid the microbial spoilage of wines. For that purpose, the inhibitory effect of pressurized extracts from six oenological woods was assessed against eleven wine microorganisms (six LAB, two AAB and three yeasts). The antioxidant activity and total phenolic content of the extracts were also evaluated to try to find a relationship with the antimicrobial effect.

2. Materials and methods

2.1. Oenological wood samples

Six commercial toasted and non-toasted oak chips samples, sized 2 × 1 × 0.1 cm, from different provenances (American (*Quercus alba*), French (*Quercus petraea*) and Rumanian (*Quercus robur*)), were supplied by the cooperage Magreñan S.L. (La Rioja Spain). Initially, oak wood samples were naturally seasoned in the open air, and part of the non-toasted oak woods were submitted to a medium-intensity toasting (45–50 min) with the temperature of wood surface being 160–170 °C. Prior to the extraction process, oak chips samples were ground with a mechanical mill and sieved (size < 1 mm) to obtain a homogenous sawdust. The sample set also included other oenological woods such as chestnut (*Castanea sativa* Mill.) and cherry *(Prunus avium* L.) grown in the north of Spain (Lugo). The samples were collected following the pattern provided by the Spanish Association for Standardisation and Certification [UNE 56-528-78]. From each tree, discs of wood were obtained at a height of 1.3 m from the base of the trunk. From each disc, samples (heartwood) measuring 20 × 20 × 40 mm were taken. The wooden blocks were dried as follows: samples were saturated with water, and then stabilised to 12% internal humidity at 20 ± 2 °C and 65% relative moisture. Finally, blocks were heated to dryness (0% internal humidity) in an oven at 103 ± 2 °C. For their analysis, the wooden blocks were ground with a mechanical mill and sieved (size < 1 mm) in order to obtain a homogenous sawdust. A sample of grape vine (*Vitis vinifera*, Chardonnay grape) was also included in this study although it is not used to carry out the ageing process. Chardonnay grape wine was grown in the Castilla La Mancha region (Spain) and was sieved to obtained a homogenous sawdust (size < 1 mm).

2.2. Pressurized liquid extraction of oenological wood samples

Pressurised liquid extraction of oenological wood samples was carried out by means of an accelerated solvent extractor ASE 200 (Dionex Corp, Sunnyuale, CA, USA). Five grams of sawdust, dispersed in 2 g of diatomaceous earth, was placed into inox extraction cells of 11 mL, which was filled with a mixture of methanol/water (50:50) as extracting solvent. One static extraction phase lasting 10 min was carried out under 1500 psi of pressure and 150 °C of temperature. Between extractions, a rinse of the complete system was performed to avoid any carry-over. All extractions were done in duplicate. An aliquot of the wood extracts were used to carry out the total phenolic content and the antioxidant capacity measurements. The rest of the extracts were frozen at −80 °C for 24 h. Then the extracts were freeze-dried in a vacuum (2.4×10^{-2} mB) at −49 °C for 24 h for the further antibacterial assays.