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Influence of phenolic compounds from wines on the growth of *Listeria monocytogenes*

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Abstract

The anti-microbial properties against *Listeria monocytogenes* of pure flavonoids rutin, catechin and quercetin; non-flavonoids gallic, vanillic, protocatechuic and caffeic acids and total polyphenols of three Argentinean wines, Cabernet Sauvignon, Malbec and Merlot varieties were investigated. The non-flavonoid caffeic acid and the flavonoids rutin and quercetin were the compounds with higher inhibitory activities on *L. monocytogenes* growth. The knowledge of the anti-listerial effect of different wines varieties could be the basis to demonstrate if the wine consumption with a meal may collaborate in the health protection against some foodborne organisms such as *L. monocytogenes.*

Keywords: Phenolic compounds; Anti-microbial activity; Wine; Growth inhibition; *Listeria monocytogenes*

1. Introduction

Wine composition depends on the grapes used to make the wine and on the vinification conditions (Cheynier, Hidalgo Arellano, Bouquet, & Mountounet, 1997). Phenolic compounds are responsible for some of the major organoleptic properties of wines, in particular color and astringency. Polyphenolic substances in wine are usually subdivided into two groups: flavonoids and non-flavonoids. The non-flavonoids in wine, phenols with only one aromatic ring, are derivatives of hydroxycinnamic acid and of hydroxybenzoic acid. The flavonoids have a common core, the flavane nucleus, consisting of two benzene rings (A and B) linked by an oxygen-containing pyrane ring (Van de Wiel, van Golde, & Hart, 2001).

Interest in phenolic compounds of grapes and wines has increased in recent years because of their potential beneficial effects on human health (Caillet, Salmiéri, & Lacroix, 2006; Frankel, Waterhouse, & Teissedre, 1995; Rodrigo & Bosco, in press; Staško, Polovka, Brezová, Biskupič, & Malí'k, 2006; Zafrilla et al., 2003). Epidemiological studies have repeatedly shown an inverse association between the risk of myocardial infarction and the consumption of wine or the intake level of some particular flavonoids, but no clear associations have found between cancer risk and polyphenols consumption (Scalbert, Manach, Morand, Rémésy, & Jiménez, 2005). Phenolic compounds are potent anti-oxidants and exhibit various physiological activities including anti-inflammatory, anti-allergic, anti-carcinogenic, anti-hypertensive, anti-arthritic and anti-microbial activities.

Phenolic compounds may affect growth and metabolism of bacteria. They could have an activating or inhibiting effect on microbial growth according to their constitution and concentration (Alberto, Farías, & Manca de Nadra, 2001, 2002; Alberto, Gómez-Cordovés, & Manca de Nadra, 2004). On pathogenic microorganisms the anti-bacterial

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effect depends of the phenolic compounds and of the strains tested. (Puupponen-Pimiä et al., 2005; Wen, Delaquis, Stanich, & Toivonen, 2003). Rodríguez Vaquero, Alberto, and Manca de Nadra (in press) reported the anti-microbial properties of pure phenolic compounds and polyphenols of different wines against *Proteus mirabilis*, *Serratia marcescens*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Staphylococcus aureus.*

The food contamination by microorganisms has attracted increased attention because it is a problem that has not yet been brought under adequate control despite the preservation techniques available. *Listeria monocytogenes* has been recognized as an emerging foodborne pathogen and has become a major concern to the food-processing industry and to health authorities over the last decades. It is found in soil, water, dairy products, including soft cheeses, and in raw and undercooked meat, poultry, seafood and related produce.

L. monocytogenes assumed public health significance as a result of its presence in foods linked to several outbreaks of listeriosis. Despite the efforts to eradicate the organism from foods, *L. monocytogenes* contamination continues to occur. It is a common bacterium in environment and animals, and may be transferred to food and human gastrointestinal tract via raw milk and contaminated dairy products. This organism may cause meningitis, sepsis or abortion, but in practice only pregnant women and people with immune defects are in danger of infection (Nester, Roberts, Pearsall, Anderson, & Nester, 1998).

Resistance to anti-microbial agents has become an increasingly important and pressing global problem. So, new classes of anti-microbial drugs are urgently required. In recent years, there has been growing interest in alternative therapies and the therapeutic use of natural products, especially those derived from plants (Rates, 2001). It is generally accepted that phytochemicals are less potent anti-infectives than agents of microbial origin, i.e. antibiotics. However, new classes of anti-microbial drug are urgently required and the flavonoids represent a novel set of leads. Future optimization of these compounds through structural alteration may allow the development of a pharmacologically acceptable anti-microbial agent or group of agents (Cushnie & Lamb, 2005).

The aim of this work was to investigate and compared the anti-microbial properties of pure flavonoid and non-flavonoid phenolic compounds and total polyphenols of three Argentinean wines varieties, Cabernet Sauvignon, Malbec and Merlot against *L. monocytogenes*.

2. Materials and methods

2.1. Microorganism and culture conditions

The bacterium used as test organism, *L. monocytogenes*, was isolated from human infection by public Hospital of Tucumán, Argentina. This bacterium was identified in our laboratory by its biochemical properties. *L. monocytogenes* was grown aerobically at 30 °C in brain heart infusion (BHI) broth and agar (Britania, Argentina) medium, pH 7.0.

Before experimental use, cultures from solid medium were sub-cultured in liquid media, incubated for 24 h and used as the source of inocula for each experiment.

2.2. Chemicals

Gallic acid was obtained from Merck (Darmstadt, Germany), catechin was obtained from Sigma (St. Louis, MO), vanillic acid, protocatechuic acid, caffeic acid, quercetin and rutin were purchased from ICN (Ohio, USA). Ciocalteu's phenol reagent and sodium carbonate were from Merck.

2.3. Samples

2.3.1. Pure phenolic compounds

For agar diffusion assays and growth curves, all phenolic compounds were dissolved in ethanol 99.8% and filter-sterilized through a 0.22 μm membrane filter.

2.3.2. Wines

Different Argentinean wines Cabernet Sauvignon, Malbec and Merlot were used. Wine samples were protected against sunlight and stored at 4°. Wines were concentrated in rotary evaporator. Without concentrate, two and four-fold concentrated (1×, 2×and 4×) wines were clarified by the addition of 30, 60 and 120 mg/ml of activated charcoal, respectively, in order to eliminate phenolic compounds. All wine samples were filter-sterilized. Clarified wines were used as controls.

2.4. Colorimetric determination of total phenolic compounds

Colorimetric determination of total phenolics was based on the procedure of Singleton and Rossi (1965). A standard curve of gallic acid was used. Results are expressed as milligram per liter gallic acid equivalents (GAE).

2.5. Anti-bacterial activity

2.5.1. Agar diffusion assay

The agar diffusion test was used to investigate anti-bacterial effects of phenolic compounds. Soft agar medium was inoculated with liquid overnight culture to a cell density of 2.0×10^9 cfu/ml, and plates containing 10 ml of agar media were overlaid with 10 ml of this inoculated soft agar. Equidistant holes were made in the agar. Thirty microlitre volumes of each sample were pipetted into the agar wells. Chloramphenicol (1000 mg/l) was used as a positive control and the negative control was ethanol. After 24 h incubation the inhibition zones were measured to an accuracy of 0.5 mm and the effect was calculated as a mean of triplicate tests.

2.5.2. Growth curves in presence of pure phenolic compounds

The liquid growth medium used in this experiment was BHI. The initial pH was adjusted to 7.0. Phenolic