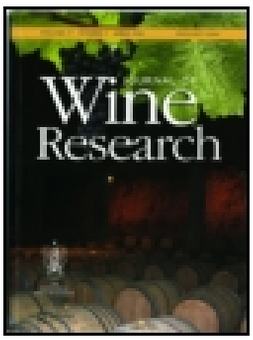

Journal of Wine Research

ISSN: 0957-1264 (Print) 1469-9672 (Online) Journal homepage: http://www.tandfonline.com/loi/cjwr20

Influence of wine phenolic compounds on viability and exopolysaccharide production by Pediococcus pentosaceus

Silvana Cecilia Ledesma, Maria Gilda Stivala, María Cristina Rubio, Martín Leandro Fanzone, Viviana Jofré & Pedro Aredes-Fernández

To cite this article: Silvana Cecilia Ledesma, Maria Gilda Stivala, María Cristina Rubio, Martín Leandro Fanzone, Viviana Jofré & Pedro Aredes-Fernández (2018): Influence of wine phenolic compounds on viability and exopolysaccharide production by Pediococcus pentosaceus, Journal of Wine Research, DOI: 10.1080/09571264.2018.1465901

To link to this article: https://doi.org/10.1080/09571264.2018.1465901

Published online: 27 Apr 2018.

JOURNAL OF WINE RESEARCH, 2018
https://doi.org/10.1080/09571264.2018.1465901

Routledge
Taylor & Francis Group

Influence of wine phenolic compounds on viability and exopolysaccharide production by *Pediococcus pentosaceus*

Silvana Cecilia Ledesma[a,b]*, Maria Gilda Stivala[a,b]*, María Cristina Rubio[a], Martín Leandro Fanzone[c], Viviana Jofré[c] and Pedro Aredes-Fernández [a,b]

[a]Instituto de Biotecnología, Facultad de Bioquímica, Química y Farmacia, Universidad Nacional de Tucumán, Tucumán, Argentina; [b]Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), San Miguel de Tucumán, Argentina; [c]Laboratorio de Aromas y Sustancias Naturales, Estación Experimental Agropecuaria Mendoza, Instituto Nacional de Tecnología Agropecuaria (INTA), Mendoza, Argentina

ABSTRACT

Low molecular weight phenolic fractions of Cabernet Sauvignon (LMF-C) and Malbec (LMF-M) wines from Colalao del Valle, Tucuman, Argentina, were isolated and chemically characterized. The effect of these phenolic fractions on viability and exopolysaccharide production of *Pediococcus pentosaceus* E2p, a wine spoilage bacterium, was examined in synthetic wine-like medium. The concentration of phenolic acids detected in LMF-C was 47.28% higher than that determined in LMF-M. The presence of LMF-C and LMF-M at concentration four times higher than detected in wine, produce a decrease in viable cells from the inoculum of 3.01 and 3.65 Log cfu mL^{-1}, respectively, and sometimes a significantly decrease in exopolysaccharide release was detected for both fractions. Considering the current trend in the search of potential and effective antimicrobial agents for total or partial replacement of sulfites in the winemaking process, these results could be of great interest, even though that phenolic compounds can also add extra value to the final product, considering their recognized beneficial properties on human health.

ARTICLE HISTORY
Received 28 August 2017
Accepted 3 April 2018

KEYWORDS
Lactic acid bacteria; *Pediococcus pentosaceus*; phenolic compounds; wine; exopolysaccharides

1. Introduction

During winemaking process, and more frequently during malolactic fermentation and ageing process, several lactic acid bacteria (LAB) mainly belonging to *Pediococcus* genus can produce alterations in the final product by overproduction of exopolysaccharides, increasing the viscosity and deteriorating the wine quality. (Lonvaud-Funel & Joyeux, 1988). In the wine industry, sulfur dioxide (SO_2) is used as an antimicrobial agent during the process of winemaking (Romano & Suzzi, 1993). However, SO_2 can cause allergic reactions in sensitive consumers (Ribéreau-Gayón, Dubourdieu, Donéche, & Lonvaud, 2006). The current trend toward a healthier lifestyle proposes alternatives to reduce or replace the use of SO_2 in wines (Guerrero & Cantos-Villar, 2015). In recent years, several authors have demonstrated that phenolic compounds (PC), natural constituents of grapes and

CONTACT Pedro Aredes-Fernández pedroaredes@fbqf.unt.edu.ar Instituto de Biotecnología, Facultad de Bioquímica, Química y Farmacia, Universidad Nacional de Tucumán, Ayacucho 491, Tucumán 4000, Argentina
*These authors contributed equally to this work and thereby both are co-first authors.

wines, have antimicrobial activity against several LAB wine species (García-Ruíz et al., 2008, 2009; Reguant, Bordons, Arola, & Rozés, 2000; Stivala et al., 2014; Stivala, Villecco, Fanzon, Jofré, & Aredes-Fernández, 2015) in addition to their beneficial effects on human health (Lin & Weng, 2006). The wines produced in Tucuman province (Argentina) have not been chemically characterized yet and they have never been assayed for their antimicrobial effect against wine spoilage bacteria. This study examines by the first time the phenolic profile of low molecular weight fraction from Malbec and Cabernet Sauvignon wines produced in Tucuman and explore the antimicrobial activity and exopolysaccharide production of these fraction on a ropy *P. pentosaceus* strain isolated from red wine.

2. Materials and methods

2.1. Samples

Two different red wines varietals corresponding to Cabernet Sauvignon and Malbec 2014 vintage were obtained directly from a local winery located at 1750 m altitude in Tucuman, Argentina.

2.2. Isolation of phenolic compound fraction

The low molecular weight fraction of phenolic compounds (LMF) was isolated using a liquid/liquid extraction method previously described by Stivala et al. (2014).

2.3. Characterization of the wine phenolic fraction

Total PC content was determined using the colorimetric method by Singleton and Rossi (1965) adapted for microtechniques by Cicco, Lanorte, Paraggio, Viggiano, and Lattanzio (2009), using gallic acid as standard. An accurately weighed portion of phenolic fraction was analyzed by HPLC-DAD in a Perkin-Elmer Series 200 (Fanzone et al., 2011). All individual PC were confirmed by HPLC-DAD/ESI-MS.

2.4. Microorganism and growth conditions

Pediococcus pentosaceus E2p was isolated from Argentinean red wine (Strasser de Saad & Manca de Nadra, 1987). Bacterial growth was performed in synthetic wine-like medium (SWM) contained 1.7% (w/v) of YNB (DifcoTM & BBLTM) and (in g L^{-1}): glucose 5.0; fructose 3.0; L-malic acid 3.0; tartaric acid 4.0; citric acid 0.7; K_2SO_4 0.1; $MgSO_4$ 0.025 and $MnSO_4$ 1.0, (pH: 4.5). The medium was supplemented with 5% ethanol (99.5% v/v) and all the essentials amino acids required by this microorganism (Aredes Fernandez, Saguir, & Manca de Nadra, 2003). The medium was sterilized by filtration (0.22 µm).

2.5. Antibacterial activity

Antibacterial activity was assayed by determination of the decrease in cell viability from the inoculum (10^6 cfu mL^{-1}) in SWM individually supplemented with LMF at two concentrations: 1×: same wine concentration and 4×: four times higher than wine. A control assay without LMF addition was carried out. At 0 and 72 h of incubation at 28°C, bacterial

viability was determined by counting of viable cells on MRS-agar medium under microaerophilic conditions. The change in viability between the start of the inoculum and at the end of the incubation time (A) was determined as follows (Equation (1)):

$$A[\text{cfu mL}^{-1}] = \text{Log}\ (x - x_0) \quad (1)$$

where x is the viable cell concentration at the end of the incubation time and x_0 the viable cell concentration at the start of the inoculum. Immediately after inoculation and after 72 h of incubation, cells and supernatant were collected by centrifugation at 10,000 × g for 20 min. at 4°C.

2.6. Electron microscopy

The cellular pellets collected (0 and 72 h) were examined by transmission electron microscopy (Carl Zeiss NTS GmbH, Oberkochen, Germany) at the Integral Electron Microscopy Center (IEMC), Tucumán, Argentina, following the procedures described by Venable and Coggeshall (1965).

2.7. Polysaccharide determination

The soluble polysaccharides in the supernatant (0 and 72 h) were precipitated with absolute ethanol and centrifuged at 14,000 × g for 10 min at 4°C. The polysaccharide concentration of the sample was determined using the phenol-sulfuric method using glucose as standard (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956).

2.8. Statistical analysis

The means and reproducibility of data were calculated based on three independent experiments performed in duplicate.

3. Results and discussion

3.1. Characterization of low molecular weight phenolic compounds in Tucuman wines

The LMF was isolated and characterized in qualitative and quantitative way from Malbec and Cabernet Sauvignon wine varietals produced in Tucuman, Argentina. The results allowed to group the PC into non-flavonoids (Table 1) and flavonoids (Table 2). The total concentration of PC in LMF-C and LMF-M, determined as the sum of the concentrations of individual compounds, was 232.60 and 294.40 mg L^{-1}, respectively. The Folin–Ciocalteu method determined a total concentration of 363.32 and 323.92 mg L^{-1} GAE in LMF-C and LMF-M, respectively. Concerning to non-flavonoids, the concentration of acids phenolic (PA) detected in LMF-C was 47.28% higher than that determined in LMF-M for Tucuman wines. In general, the characterization of LMF in both studied varietals was comparable to data reported by other authors using similar extraction procedures (Fanzone et al., 2011; Ghiselli, Nardini, Baldi, & Scaccini, 1998; La Torre, Saitta, Vilasi, Pellicano, & Dugo, 2006; Stivala et al., 2014, 2015). The concentration of PA

Table 1. Quantification of non-flavonoid phenolic compounds in the low molecular weight fraction of Cabernet Sauvignon (LMF-C) and Malbec (LMF-M) wines.

Non-flavonoid compounds		
	Concentration [mg L^{-1}]	
Compounds	LMF-C	LMF-M
Hydroxybenzoic acids		
Gallic acid	13.1 ± 1.2	9.6 ± 0.8
Protocatechuic acid	2.0 ± 0.2	0.7 ± 0.06
Syringic acid	1.8 ± 0.1	1.8 ± 0.1
Ethyl gallate	2.8 ± 0.2	1.8 ± 0.1
Methyl gallate	6.3 ± 0.6	4.3 ± 0.4
Total	26.0 ± 2.2	18.2 ± 1.7
Hydroxycinnamic acids		
trans-caftaric acid	6.7 ± 0.6	4.6 ± 0.4
cis-caftaric acid	4.1 ± 0.4	2.1 ± 0.2
trans- coutaric acid	2.8 ± 0.2	2.6 ± 0.2
trans-caffeic acid	8.9 ± 0.8	4.9 ± 0.4
trans-fertaric acid	3.2 ± 0.3	2.4 ± 0.2
p-coumaric acid	5.5 ± 0.5	3.9 ± 0.3
Total	**31.2 ± 2.5**	**20.5 ± 2.1**
Total phenolic acids	57.2 ± 2.3	38.7 ± 1.9
Phenolic alcohols		
Tyrosol	26.7 ± 2.6	12.7 ± 1.3
Stilbenes		
trans-resveratrol glucoside	0.8 ± 0.1	3.6 ± 0.35
Total non-flavonoids	84.7 ± 8.3	55.0 ± 4.7

*Values are expressed as mean ± standard deviation.

in the LMF-C was similar to that reported for the same varietal produced in Salta-Argentine (Stivala et al., 2014, 2015), and 15.91% higher respect to the same varietal produced in Mendoza (Fanzone et al., 2011). The PA concentration of LMF-M was 41.80% lower and 26.30% higher than the same varietals produced in Salta and Mendoza, respectively (Fanzone et al., 2011; Stivala et al., 2015). With respect to hydroxybenzoic acids, the total concentration in LMF-C was 42.90% higher than that detected in LMF-M. In agreement with other studies (Fanzone et al., 2012; Stivala et al., 2014, 2015), gallic acid was the major compound detected in both fractions, being 36.50% higher in LMF-C than in LMF-M. The total hydroxycinnamic acid concentration was 52.20% higher in LMF-C than in LMF-M. The concentration of these compounds in LMF-M were 50% lower than that reported for the same fraction isolated from Salta-Argentine wine (Stivala et al., 2014, 2015), but in similar concentration than that reported in Mendoza wines (Fanzone et al., 2011).

Between the hydroxycinnamic acids, the main compound detected was *trans*-caffeic acid. In the LMF-C, this compound was higher than in LMF-M in 81.60%. The caffeic acid concentration detected in both studied varietals was higher than that reported for Salta and Mendoza wines (Fanzone et al., 2011; Stivala et al., 2014, 2015). Flavanols compounds were the main constituents detected within flavonoids, being (-)-epicatechin and the procyanidin dimer the most abundant molecules in both fractions. The flavonoids and non-flavonoids compounds were detected at similar concentrations in same wine varietals from Salta (Stivala et al., 2014, 2015).

Table 2. Quantification of flavonoid phenolic compounds in the low molecular weight fraction of Cabernet Sauvignon (LMF-C) and Malbec (LMF-M) wines.

Flavonoid compounds		
	Concentration [mg L^{-1}]	
Compounds	LMF-C	LMF-M
Flavanols		
(+)-Catechin	2.5 ± 0.2	16.4 ± 1.5
(−)-Epicatechin	40.0 ± 4.0	28.8 ± 2.7
Procyanidin dimer	40.9 ± 4.0	31.3 ± 3.1
Procyanidin trimer 1	11.5 ± 1.1	10.8 ± 1.0
Procyanidin trimer 2	8.8 ± 0.8	8.2 ± 0.8
Procyanidin trimer 3	9.3 ± 0.9	19.3 ± 1.8
Procyanidin trimer 4	3.2 ± 0.3	10.8 ± 1.0
Total	116.2 ± 11.0	125.6 ± 12.4
Flavonols		
Myricetin-3-glucuronide	2.5 ± 0.2	3.3 ± 0.3
Myricetin-3-glucoside	1.5 ± 0.1	4.3 ± 0.4
Quercetin-3-glucuronide	5.3 ± 0.5	4.1 ± 0.4
Quercetin-3-galactoside	1.4 ± 0.1	2.8 ± 0.2
Quercetin-3-glucoside	2.6 ± 0.2	6.8 ± 0.6
Quercetin-3-rhamnoside	1.5 ± 0.1	1.7 ± 0.1
Isorhamnetin-3-glucoside	10.3 ± 1.3	6.1 ± 0.6
Naringenin	3.8 ± 0.3	3.6 ± 0.3
Syringetin-3-glucoside	1.1 ± 0.1	1.2 ± 0.1
Total	30.0 ± 3.1	33.9 ± 3.4
Dihydroflavonols		
Dihydroquercetin-3-glucoside	nd	31.6 ± 3.1
Dihydroquercetin-3-rhamnoside	1.7 ± 0.1	3.3 ± 0.3
Total	1.7 ± 0.1	34.9 ± 3.2
Total flavonoids	147.9 ± 14.5	194.4 ± 19.5
Total phenolic compounds	232.6 ± 22.9	249.4 ± 24.7

*Values are expressed as mean ± standard deviation; nd: not detected.

3.2. Effect of the phenolic fraction on growth and cell integrity of P. pentosaceus

Table 3 shows that LMF-C and LMF-M at concentration 1× produced a reduction about 1 Log cycle in viable cell count after 72 h of incubation in SWM. The addition of either LMF four times concentrated to SWM produce a substantial drop in viable cells count (3 Log cycle) after 72 h incubation. The cellular integrity of *P. pentosaceus* E2p in SWM medium without addition of LMF remain unaltered after 72 h incubation (Figure 1(a)). Incubation in SWM supplemented with LMF-M and LMF-C at concentration 4× (Figure 1(b,c), respectively) showed modifications in the cell morphology with alterations in the cell integrity of the microorganism. Pure PA present in wine exerts antimicrobial activity against wine LAB (García-Ruíz et al., 2008; Stead, 1993). Few studies have been performed to assess the antimicrobial effect of phenolic extracts on LABs. García-Ruiz et al. (2012) reported

Table 3. Growth parameters of *P. pentosaceus* E2p in SWM individually supplemented with different LMF obtained from Tucuman wines.

Culture medium	A [Log cfu mL^{-1}]
SWM	0.690 ± 0.06
SWM + LMF-C 1X	−1.180 ± 0.16
SWM + LMF-C 4X	−3.010 ± 0.31
SWM + LMF-M 1X	−1.240 ± 0.22
SWM + LMF-M 4X	−3.650 ± 0.34

*Values are expressed as mean ± standard deviation.

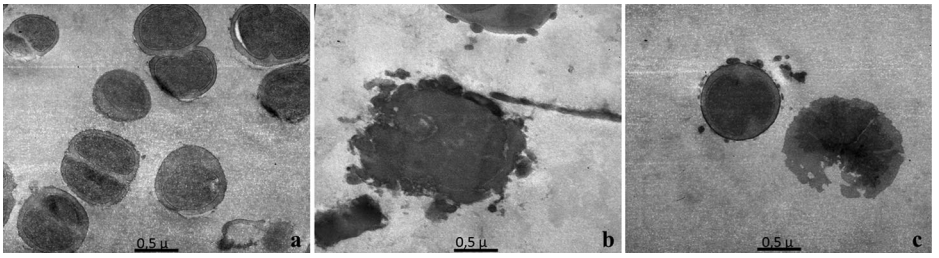

Figure 1. Electron micrographs of ultrathin sections of *P. pentosaceus* E2p obtained after 72 h of incubation in SWM (20,000×) (a), in SWM supplemented with a fourfold concentrated fraction (4×) of phenolic compounds of low molecular weight from Malbec wine (30,000×) (b), and in SWM supplemented with a fourfold concentrated fraction (4×) of phenolic compounds of low molecular weight from Cabernet Sauvignon (20,000×) (c).

antimicrobial activity against wine LABs (*L. hilgardii*, *L. plantarum*, *L. casei*, *P. pentosaceus* and *O. oeni*) of commercial plant extracts from the diverse origin, performing those experiences in complex culture medium and using high concentrations of extracts, ranging from 330 to 2800 mg L^{-1} GAE. We perform the assays in a synthetic medium similar to wine, and wine extracts were assayed at concentrations ranging from 300 to 1200 mg L^{-1}. On the basis of previous knowledge, the antibacterial effect observed of LMF in SWM can be attributed to the presence of PA of low molecular weight and more specifically to presence of caffeic acid (Campos et al., 2009; García-Ruiz, Bartolomé, Cueva, Martín-Álvarez, & Moreno-Arribas, 2009), which was found in high concentrations in LMF. From the electron microscopy results, is possible to infer that the bacterial inhibitory activity is associated with alterations in cell integrity. Several authors have reported that polyphenols alter the structure of the bacterial cell membrane causing the escape of intracellular constituents (Johnston,

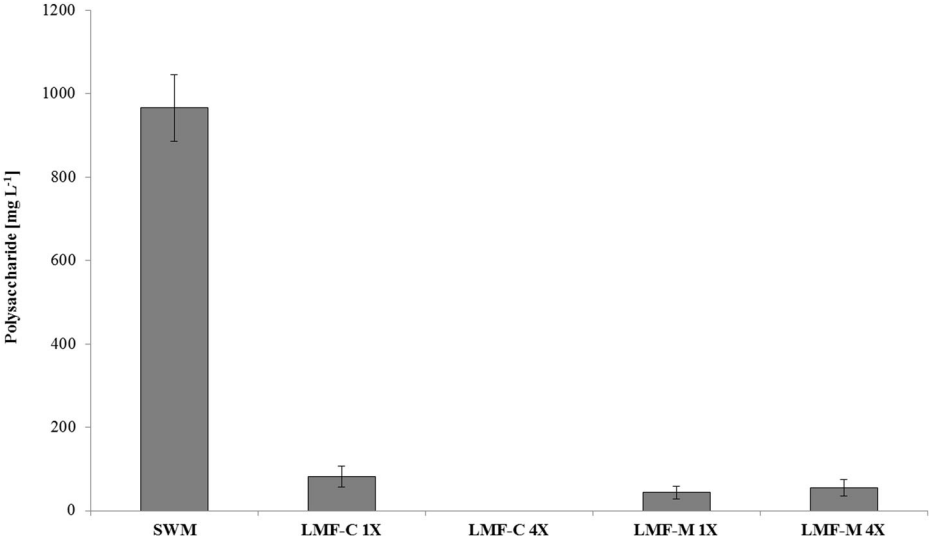

Figure 2. Effect of two different concentrations (1× and 4×) of LMF-C and LMF-M from wines varietals corresponding to Cabernet Sauvignon and Malbec on exopolysaccharide production by *P. pentosaceus* E2p in SWM.

Hanlon, Denyer, & Lambert, 2003; Rodríguez et al., 2009), moreover there are evidence that PA were able to enhance influx of protons and efflux of potassium and phosphate in *O. oeni* and *L. hilgardii* cell suspensions (Campos et al., 2009).

3.3. Effect of phenolic compounds on bacterial exopolysaccharide production

In SWM, *P. pentosaceus* E2p produced 966.07 mg L^{-1} of extracellular exopolysaccharide. Figure 2 shows that in presence of both LMF studied, a diminution in exopolysaccharide production was detected. The supplementation with a fourfold concentrated LMF to SWM, produced a decrease in exopolysaccharide production higher than 95%, being undetectable the exopolysaccharide concentration in SWM in presence of LMF-C. Consistent with the results of Stivala et al. (2015) on *P. pentosaceus* 12p, the LMF produce a marked decrease in bacterial polysaccharide production in SWM.

This is the first time that the antimicrobial effect of low molecular weight phenols extracted from Tucuman wines were assayed against a wine spoilage lactic acid bacterium. The use of PC could be promising for the search of new antimicrobial agents as an effective alternative to the use of sulfites in enology to control of spoilage wine bacteria.

Acknowledgments

The authors wish to thank CONICET and CIUNT.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work was supported by Agencia Nacional de Promoción Científica y Tecnológica: [Grant Number Project 2016 N°4045]; Consejo Nacional de Investigaciones Científicas y Técnicas [Grant Number Project N°0627].

ORCID

Pedro Aredes-Fernández http://orcid.org/0000-0001-7130-4245

References

Aredes Fernandez , P. A., Saguir, F. M., & Manca de Nadra , M. C. (2003). Effect of amino acids and peptides on growth of *Pediococcus pentosaceus* from wine. *Latin American Applied Research*, *33*, 135–139.

Campos, F. M., Couto, J. A., Figueiredo, A. R., Tóth, I. V., Rangel, A. O., & Hogg, T. A. (2009). Cell membrane damage induced by phenolic acids on wine lactic acid bacteria. *International Journal of Food Microbiology*, *135*, 144–151.

Cicco, N., Lanorte, M. T., Paraggio, M., Viggiano, M., & Lattanzio, V. (2009). A reproducible, rapid and inexpensive Folin–Ciocalteu micro-method in determining phenolics of plant methanol extracts. *Microchemical Journal*, *91*, 107–110.

Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., & Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Analytical Chemistry*, *28*, 350–356.

Fanzone, M., Peña-Neira, A., Gil, M., Jofré, V., Assof, M., & Zamora, F. (2012). Impact of phenolic and polysaccharidic composition on commercial value of Argentinean Malbec and Cabernet Sauvignon wines. *Food Research International*, *45*, 402–414.

Fanzone, M., Zamora, F., Jofré, V., Assof, M., Gómez Cordovés, C., & Peña Neira, A. (2011). Phenolic characterization of red wines from different grape varieties cultivated in Mendoza province (Argentina). *Journal Science Food Agriculture*, *24*, 154–159.

García-Ruiz, A., Bartolomé, B., Cueva, C., Martín-Álvarez, P. J., & Moreno-Arribas, M. V. (2009). Inactivation of oenological lactic acid bacteria (*Lactobacillus hilgardii* and *Pediococcus pentosaceus*) by wine phenolic compounds. *Journal of Applied Microbiology*, *107*, 1042–1053.

García-Ruiz, A., Cueva, C., González-Rompinelli, E. M., Yuste, M., Torres, M., Martín-Álvarez, P. J., … Moreno-Arribas, M. V. (2012). Antimicrobial phenolic extracts able to inhibit lactic acid bacteria growth and wine malolactic fermentation. *Food Control*, *28*, 212–219.

García-Ruíz, A., Bartolomé, B., Martínez-Rodríguez, A. J., Pueyo, E., Martín-Álvarez, P. J., & Moreno-Arribas, M. V. (2008). Potential of phenolic compounds for controlling lactic acid bacteria growth in wine. *Food Control*, *19*, 835–841.

Ghiselli, A., Nardini, M., Baldi, A., & Scaccini, C. (1998). Antioxidant activity of different phenolic fractions separated from an Italian red wine. *Journal of Agricultural and Food Chemistry*, *46*, 361–367.

Guerrero, R. F., & Cantos-Villar, E. (2015). Demonstrating the efficiency of sulphur dioxide replacements in wine: A parameter review. *Food Science and Technology*, *42*, 27–43.

Johnston, M. D., Hanlon, G. W., Denyer, S. P., & Lambert, R. J. W. (2003). Membrane damage to bacteria caused by single and combined biocides. *Journal of Applied Microbiology*, *94*, 1015–1023.

La Torre , G. L., Saitta, M., Vilasi, F., Pellicano, T., & Dugo, G. (2006). Direct determination of phenolic compounds in Sicilian wines by liquid chromatography with PDA and MS detection. *Food Chemistry*, *94*, 640–650.

Lin, J. K., & Weng, M. S. (2006). Flavonoids as nutraceuticals. In E. Grotewold (Ed.), *The science of flavonoids* (pp. 213–238). New York: Springer.

Lonvaud-Funel, A., & Joyeux, A. (1988). A bacterial disease causing ropiness of wines. *Science des Aliments*, *8*, 33–50.

Reguant, C., Bordons, A., Arola, L., & Rozés, N. (2000). Influence of phenolic compounds on the physiology of *Oenococcus oeni* from wine. *Journal of Applied Microbiology*, *88*, 1065–1071.

Ribéreau-Gayón J., Dubourdieu D., Donéche B. & Lonvaud A. (2006). *Handbook of enology: Red winemaking* (pp. 327–385). Chichester: John Wiley & Sons, Ltd.

Rodríguez, H., Curiel, J. A., Landete, J. M., de las Rivas, B., de Felipe, F. L., Gómez-Cordovés, C., … Muñoz, R. (2009). Food phenolics and lactic acid bacteria. *International Journal of Food Microbiology*, *132*, 79–90.

Romano, P., & Suzzi, G. (1993). Sulfur dioxide and wine microorganisms. In G. H. Fleet (Ed.), *Wine microbiology and biotechnology* (pp. 373–393). Switzerland: Harwood Academic Publishers.

Singleton, V. L., & Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture*, *16*, 144–158.

Stead, D. (1993). The effect of hydroxycinnamic acids on the growth of wine-spoilage lactic acid bacteria. *Journal of Applied Bacteriology*, *75*, 135–141.

Stivala, M. G., Villecco, M. B., Fanzon, M., Jofré, V., & Aredes-Fernández, P. (2015). Characterization of the phenolic fraction from Argentine wine and its effect on viability and polysaccharide production of *Pediococcus pentosaceus*. *Biotechnology Letters*, *37*, 2435–2444.

Stivala, M. G., Villecco, M. B., Fanzone, M., Jofré, V., Rodríguez-Vaquero, M. J., & Aredes-Fernández, P. (2014). Characterization, antibacterial and biological activities of phenolic fraction of Argentinean red wines. *The Open Conference Proceedings Journal*, *5*, 29–37.

Strasser de Saad , A. M., & Manca de Nadra , M. C. (1987). Isolation and identification of the lactic acid bacteria from Cafayate (Argentina) wines. *Microbiologie Aliments Nutrition*, *5*, 45–49.

Venable, J. R., & Coggeshall, R. (1965). A simplified lead citrate stain for use in electron microscopy. *The Journal of Cell Biology*, *25*, 407–408.