Research Note

Influence of Phenolic Compounds on Activity of Nisin and Pediocin PA-1

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Abstract: Bacteriocins have been evaluated as biopreservatives in wine. However, in red wine the phenolic compounds might have negative effects on the activity and stability of bacteriocins and consequently limit the application of bacteriocins in the wine. This study investigated the influence of phenolic compounds and polyphenols on the activity of nisin and pediocin PA-1. No influence on the bacteriocin activity was detected. Furthermore, synergistic effects between phenolic compounds and bacteriocins on the survival of *Pediococcus pentosaceus* NCDO 813 were observed. Results showed that the combination of bacteriocin with phenolic compounds or polyphenols decreased bacterial cell numbers between 3 and 6 log units.

Key words: bacteriocins, phenolic compounds, lactic acid bacteria, wine

Bacteriocins are bacterially produced antimicrobial peptides with narrow or broad host ranges (Klaenhammer 1988). Many bacteriocins are produced by food-grade lactic acid bacteria (LAB), which can inhibit or prevent the development of specific bacterial species in beverages and food. Bacteriocins can be divided in three major classes (Diep and Nes 2002). Nisin belongs to class I, the lantibiotics, and is produced by some strains of *Lactococcus lactis* (De Vuyst 1994, Delves-Broughton 2005). It is one of the most industrially relevant bacteriocins and has been used for decades in many countries as a safe and effective food preservative (Delves-Broughton 2005). Pediocin PA-1 belongs to class II and is a small heat-stable bacteriocin produced by *Pediococcus acidilactici* PAC1.0 (Marugg et al. 1992). Nisin (Rojo-Bezares et al. 2007) and pediocin PA-1 (Du Toit 2002) have been shown to inhibit spoilage bacteria, including lactic acid bacteria found in wine such as *Lactobacillus plantarum, L. paracasei*, *L. brevis*, *L. hilgardii, L. pentosus, Leuconostoc mesenteroides, Pediococcus pentosaceus,* and *Oenococcus oeni*. Moreover, it was demonstrated that these peptides are stable under winemaking conditions and have no negative effect on either yeast growth or wine sensory profile.

The possibility of controlling bacterial growth during vinification and preservation by bacteriocins is a promising alternative to meet consumer demands and preferences of minimally processed products that contain less chemical preservatives such as sulfur dioxide (SO_2). Nevertheless, although total SO_2 could be lowered with the use of bacteriocins, a certain free SO_2 content in wine is still required for antioxidant and antioxidasic purposes. Bacteriocins could be used to promote quality by inhibiting indigenous LAB microflora, thereby preventing the production of undesired compounds such as off-flavors and biogenic amines. Consequently, malolactic fermentation (MLF) could be conducted with a selected starter culture. Furthermore, suitable combinations of nisin and SO_2 could control the growth of spoilage bacteria in wine, which thus allows a decrease in the amounts of SO_2 (Rojo-Bezares et al. 2007).

However, in one report a decrease in nisin activity to less than 90% was observed in Pinot noir over a 4-month storage period, while little decrease was observed in Chardonnay (Daeschel et al. 1991). These authors suggested that nisin may be interacting with polyphenolic compounds that are present in red wines but absent in white wines. A later study verified that tannins caused an immediate decrease of nisin levels when tested in a wine model system (Daeschel and Bower 1991–1992). Grapes and wine contain a wide variety of phenolic compounds that originate either from initial grape material or from wood used during maturation (oak barrels, oak chips). Red wines contain numerous phenolic compounds (De Beer et al. 2002) such as phenol acids (240 to 500 mg/L), including gallic and *p*-coumaric acid; anthocyanins (40 to 470 mg/L); flavonols (65 to 240 mg/L); and flavan-3-ols (25 to 560 mg/L), including catechin. Tannins are formed from the polymerization of elementary molecules with phenolic functions and are divided in two groups: hydrolyzable tannins and condensed tannins. Hydrolyzable tannins include gallotannins and ellagitannins and are not naturally found in grapes but in wood. Condensed tannins in grapes are basically complex polymers of flavan-3-ols or catechins. Polyphenols, tannins in particular, are capable of forming stable combinations with proteins.

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Various chemical and physical factors, such as the amino acid composition of the protein, pH, and temperature, may affect the formation of tannin-protein complexes (Ribéreau-Gayon et al. 2000). Bacteriocins could potentially be bound by polyphenols, since they are peptides.

For the possible use of bacteriocins to control malolactic fermentations, a loss or decrease in bacteriocin activity in red wines from the interaction between polyphenols and bacteriocins would therefore be disadvantageous. The purpose of this study was to investigate the short-term influence of phenolic compounds on nisin and pediocin to better understand the factors which contribute to the decrease of bacteriocin activity.

Materials and Methods

Bacterial strains and culture conditions. The bacterial strain used as sensitive test organism was *P. pentosaceus* NCDO 813 (National Collection of Dairy Organisms, Reading, UK). *Pediococcus acidilactici* PAC1.0 was used for production and purification of pediocin PA-1. Both bacteria strains were grown at 30°C in MRS broth (Biolab, Merck, South Africa). A synthetic wine medium (SWM) (Ugliano et al. 2003) containing 14% v/v ethanol and pH 3.5 was used as model wine for the experiments, without the glycosidic extract.

Purification of pediocin PA-1. *Pediococcus acidilactici* PAC1.0 was cultured in 5 mL MRS broth at 30°C overnight and then reinoculated into 1-L MRS broth at 30°C overnight. Pediocin PA-1 was isolated from the culture by harvesting the cells (8000 x *g*, 10 min, 4°C). Solid ammonium sulfate (85% of saturation) was added to the cell-free supernatant to precipitate the protein and stirred at 8°C over 8 hr. The precipitate was then pelleted by centrifugation (10000 x *g*, 10 min, 4°C), dissolved in deionized water, and dialyzed against deionized water for 48 hr. The dialyzed sample was freeze-dried, and the arbitrary activity unit (AU) per mL of the powder was determined by a two-fold dilution series. One arbitrary AU was defined as the reciprocal of the highest dilution showing a clear inhibition zone and was multiplied by a factor of 100 to obtain AU/mL (Du Toit et al. 2000).

Preparation of synthetic wine medium. Two phenolic acids, two flavan-3-ols, grape tannins, and oak tannins were added individually or in combinations to aliquots of SWM (see Table 1), after being dissolved in 96% ethanol. The grape tannins (VR Tannin Supra) were purchased from Laffort (Stellenbosch, South Africa) and the oak tannins (Oenotan Selection) were from Columbit (Maitland, South Africa). *p*-Coumaric acid (C9008), gallic acid (G7384), (+)-catechin (C1251), and (-)-epi-catechin (E1753) were obtained from Sigma-Aldrich (Johannesburg, South Africa).

The concentration of each component used in the synthetic wine medium was similar to the average concentration found in a red wine. Nisin (N5764, Sigma-Aldrich) and pediocin PA-1 were added to each sample at a concentration of 12,800 AU/mL. Pediocin PA-1 was only tested in combination with *p*-coumaric acid, catechin, and the oak and grape tannins. The model wine was inoculated with an overnight culture to ~10^8 cfu/mL of *P. pentosaceus* NCDO 813 and incubated at 30°C.

Detection of antimicrobial activity. Bacteriocin activity was determined by measuring the cell density of the sensitive organism. Samples were taken at specific time intervals (immediately after inoculation and after 3, 6, 9, and 24 hr) and plated out in serial dilutions on MRS agar plates in duplicate. The plates were incubated at 30°C for 24 to 48 hr.

Results

Six phenolic compounds of grapes and wine were tested individually and in combination in a model wine medium. Synthetic wine medium inoculated with the sensitive organism but without any phenolic compounds or bacteriocins served as the positive control.

Activity in presence of one phenolic compound. Both nisin and pediocin in combination with one single phenolic compound increased the inhibitory effect on the sensitive organism (Figure 1, Figure 2). The combinations with pediocin showed the strongest negative influence as the cell numbers decreased by ~10^6 cfu/mL, compared with the combination with nisin that reduced cell numbers by ~10^5 cfu/mL. The close parental relationship of the pediocin-producing strain and sensitive organism might explain this phenomenon. A specific bacterium exhibits high sensitivity to the bacteriocin produced by a closely related species and genera (Klaenhammer 1988). Similar results were obtained by combining nisin with gallic acid or catechin (data not shown).

Nisin and pediocin combined with tannins. The oak tannins in combination with nisin or pediocin had the greatest influence on the sensitive organism and almost completely inhibited survival (Figure 3). Similar results

Table 1 Concentration of phenolic compounds tested with nisin or pediocin PA-1 in a synthetic wine medium.

Code	Compound	Concn (mg/L)
co	Control (no compound added)	-
cou	*p*-Coumaric acid	200
ga	Gallic acid	200
cat	Catechin	200
epi-cat	epi-Catechin	200
gtan	Grape tannins	100
otan	Oak tannins	100
cou + ga	*p*-Coumaric acid + gallic acid	100 + 100
cou + cat	*p*-Coumaric + catechin	100 + 100
cou + epi-cat	*p*-Coumaric + epi-catechin	100 + 100
ga + epi-cat	Gallic acid + epi-catechin	100 + 100
ga + cat	Gallic acid + catechin	100 + 100
cat + epi-cat	Catechin + epi-catechin	100 + 100
ga + cou + cat + epi-cat	Gallic acid + *p*-coumaric acid + catechin + epi-catechin	50 + 50 + 50 + 50

were obtained by using grape tannins (data not shown). Results showed that the tannins alone decreased the cell numbers by 10^1 to 10^2 cfu/mL compared with the bacteriocin alone and the combinations that reduced the cell numbers by 10^3 to 10^6 cfu/mL. It was also evident that nisin alone was less effective than the nisin combinations.

Nisin combined with phenolic compounds. Nisin activity was not influenced by the addition of *p*-coumaric acid and gallic acid or *p*-coumaric acid and catechin. The combined phenolic compounds without nisin decreased the survival of the sensitive organism by ~10^3 cfu/mL, and the addition of nisin to the mixtures increased the inhibitory effect on the survival by ~10^5 cfu/mL (Figure 4).

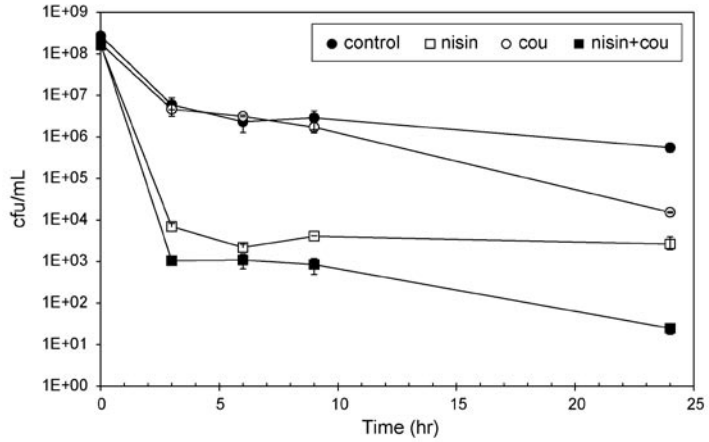

Figure 1 Effect of *p*-coumaric acid (cou) and nisin on the growth of *P. pentosaceus* NCDO 813 (see Table 1 for abbreviation codes).

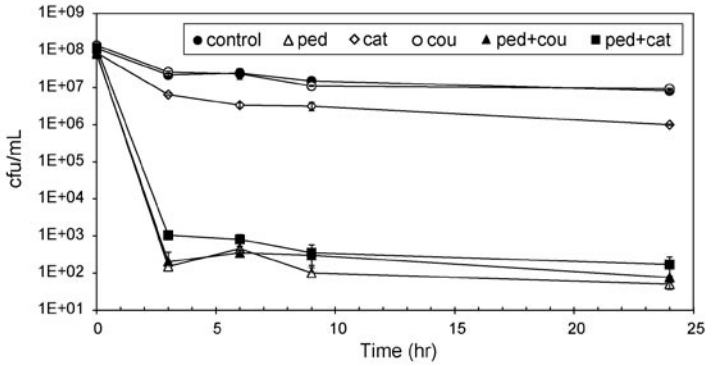

Figure 2 Effect of *p*-coumaric acid (cou), catechin (cat), and pediocin (ped) on the growth of *P. pentosaceus* NCDO 813.

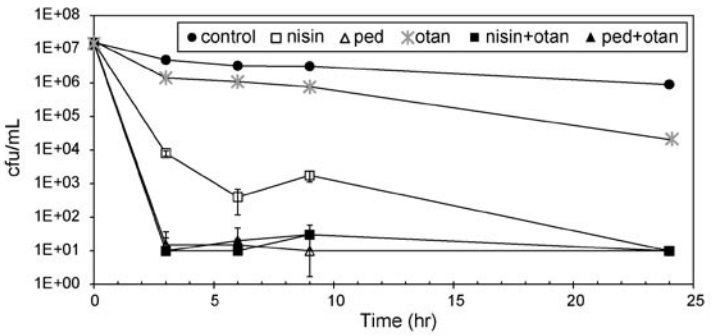

Figure 3 Effect of oak tannins (otan), nisin, and pediocin (ped) on the growth of *P. pentosaceus* NCDO 813.

The combination of gallic acid and epi-catechin did not affect the survival of the sensitive organism, whereas the combination of *p*-coumaric acid and epi-catechin as well as the mixture of phenolic compounds with nisin showed a strong inhibitory effect on the survival of *P. pentosaceus* NCDO 813. In both latter cases the cell numbers decreased by ~10^6 cfu/mL (Figure 5). The combination of the four compounds *p*-coumaric acid, gallic acid, catechin, and epi-catechin, the combination of gallic acid and catechin, and the combination of catechin and epi-catechin had little effect on the sensitive organisms. Nisin with the four compounds in combination had the strongest inhibitory effect on the survival of the sensitive organism and reduced the cell numbers by ~10^5 cfu/mL. Any combination of two compounds with nisin decreased cell numbers by ~10^4 cfu/mL (data not shown).

Discussion

Phenolic compounds are known to either stimulate or inhibit the growth and metabolism of bacteria (Campos et al. 2003, Figueiredo et al. 2008, Reguant et al. 2000, Rozès et al. 2003, Vaquero et al. 2007, Vivas et al. 2000). Their positive or negative influence depends on bacterial species, the specific phenolic acid used and its concentration, and its chemical structure (Alberto et al. 2001,

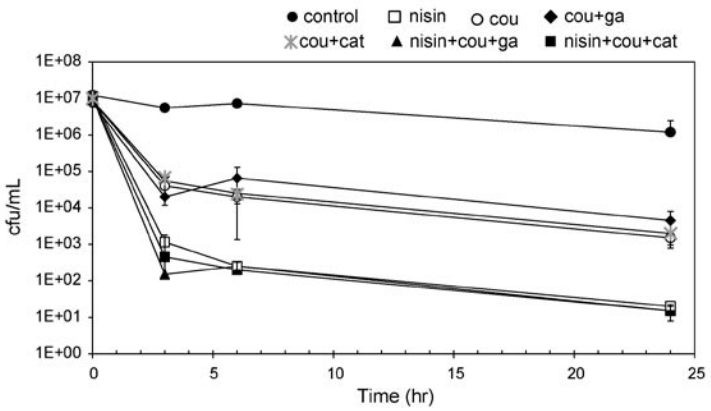

Figure 4 Effect of nisin, *p*-coumaric acid (cou), and gallic acid (ga) and of nisin, *p*-coumaric acid, and catechin (cat) on the growth of *P. pentosaceus* NCDO 813.

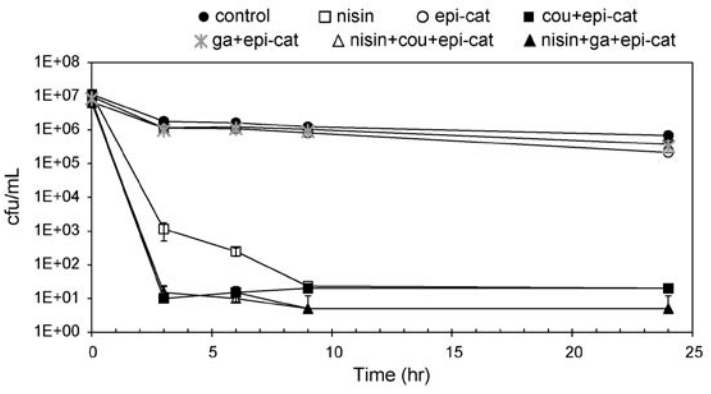

Figure 5 Effect of nisin, *p*-coumaric acid (cou), and epi-catechin (epi-cat) and of nisin, gallic acid (ga), and epi-catechin on the growth of *P. pentosaceus* NCDO 813.

2002, Figueiredo et al. 2008, Reguant et al. 2000, Vivas et al. 1997, 2000). Little is known about the interactions of phenolic compounds and bacteriocins.

In this study most of the phenolic compounds tested had a negative influence on the bacteria, and the combination of phenolic compounds and bacteriocin increased the inhibitory effect in the first 3 hours following the bacteriocin addition. In previous studies, nisin activity remained stable in white wines but decreased in red wines over a 4-month period. Moreover, a decrease in nisin activity was observed with grape tannins but not with catechin or gallic acid over a 6-week period (Daeschel and Bower 1991-1992); in addition, the activity of nisin decreased more rapidly in mature wines than in younger wines. In our study the activity of nisin and pediocin PA-1 was not inhibited by the phenolic compounds tested over a 24-hr period. Moreover, synergetic effects were observed, and after 24 hr only 5% of the bacteria survived.

The purpose of this study was to investigate the short-term influence of phenolic compounds on the activity of nisin and pediocin. Only one LAB species was used in our model system to test our hypothesis. The duration of the experiments might not have been long enough to have a negative effect on the activity and stability of the bacteriocins. Previous studies observed that not only wine pH but also storage temperature and age had an effect on the activity of nisin. Time seems to be an important factor for the inhibition of bacteriocins in wine. Therefore the use of bacteriocins might be an alternative tool to control fermentations. Finally, when studying the effects of phenolic compounds on bacteriocins, it would be important to consider the presence of other compounds in wine, such as proteins or sugars, that can interact with phenolic compounds and bacteriocins, affecting their structure and activity.

Conclusion

In this study, results showed no negative effect of phenolic compounds and polyphenols on nisin and pediocin activity. Considering the advantages of natural inhibitors versus classical chemical control methods, bacteriocins present a beneficial and more ecologically friendly alternative or might possibly replace a portion of the sulfur dioxide, once interactions with wine are unraveled.

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