P-02-16 **5:30 PM**

Antimicrobial effect of combination of phenolic acids and bacteriocins against *Salmonella* in meat products (#279)

Panagiotis Chanos, Anna M. Erdwiens, Corinna Stuehmeier, Christian Hertel

German Institute of Food Technologies, Biotechnology, Quakenbrück, Germany

Introduction

Food relevant Gram-negative bacteria are resistant to known natural antimicrobials used in the industry mainly due to the structure of their cell wall. The cell wall of Gram-negative bacteria is fortified with an outer membrane predominantly composed by lipopolysaccharides which are stabilized with bonds of divalent cations and confers additional resistance against certain antimicrobials. It is known that destabilization of the membrane by chelation of divalent cations with EDTA for example can greatly improve the impact of antimicrobial molecules, like bacteriocins, on Gram-negative bacteria. However natural chelators of Ca^{2+} and Mg^{+2} with good chelating activity at near neutral pH have not been described.

Published research has shown that some phenolic acids can destabilize the outer membrane of Gram-negative bacteria by chelation of divalent cations. As an example, 3,4-dihydroxyphenylacetic acid and 3-hydroxyphenylacetic acid increased the susceptibility of *S. enterica* subsp. *enterica* ser. Typhimurium strains to the hydrophobic antibiotic novobiocin. The purpose of this study was to investigate the potential of several phenolic acids to enhance the activity of natural antimicrobial peptide Nisin against several *Salmonella* and *E. coli* strains in vitro and at different food relevant pH. A further goal was to investigate the activity of favorable mixtures of Nisin and phenolic acids against *Salmonella* in a raw fermented sausage model.

Methods

The antimicrobial activity of different phenolic acids and Nisin against *Salmonella* and *E. coli* strains was evaluated with the microdilution assay in 96-well microtiter plates at pH 5, 5.5 and 6. The antimicrobial activity was expressed as Minimum Inhibitory Concentrations (MIC). Furthermore, the combinatory antimicrobial effects of Nisin with different phenolic acids against *Salmonella* and *E. coli* strains was investigated at pH 5.5 using the Fractional Inhibitory Concentration (FIC) assay. All *in-vitro* assays were done in biological triplicates.

The effectiveness of the antimicrobial mixtures *in-situ* was evaluated in a meat batter for traditional German raw fermented sausages (Zwiebelmettwurst) containing 100% pork meat with fat concentration of approx. 5% (w/w), curing salt (2.8% w/w), spice mix (1.2% w/w) and commercial starter culture. The batter was inoculated with a cold adapted *Salmonella* strain cocktail at 10^6 CFU/g. The fermentation of was done in plastic cups with screw lids (30 g) at 24 °C for 24 hours followed by storage of 4°C for up to 14 days. Microbiological analysis was done using a modification of the ISO 6579:2002 MPN method and in triplicate. Sampling for microbiological analysis was done at t=0 h and at t = 24 h of fermentation and thereafter at regular intervals during the cold storage. Positive (no antimicrobial but with *Salmonella*) and negative controls (no antimicrobial and no *Salmonella*) were included. Measurement of pH was done on all samples taken for microbiological analysis.

Results

Statistically significant differences (p=0.05) of the MICs of different phenolic acids were shown against the same target microorganisms. The activities were pH dependent *i.e.* lower pH increased the activity against all target strains. In general, 3-phenylpropionic acid was the most active of the phenolic acids tested. Also *S.* Typhimurium exhibited higher tolerance than *S.* Enteritidis against all phenolic acids tested. Furthermore, *E. coli* was in general more susceptible to the phenolic acids tested than *Salmonella*. Nisin exhibited little to no activity against *Salmonella* depending on the pH. A slight synergistic activity was detected between 3-phenylpropionic acid and Nisin. The combination of 3-phenylpropionic acid and Nisin at three different concentrations and at pH 5.5 was used in the meat model against a pool of *Salmonella*. A reduction of approx. 1.5 log10 MPN/g in *Salmonella* counts compared to the initial counts and an approx. 1 log10 MPN/g difference compared to the positive control was detected within the first 2 hours of fermentation at 24 °C. A linear reduction of *Salmonella* counts was observed in the samples with the highest antimicrobial concentration which over the 14-day shelf life exceeded 3 log10 MPN/g. The pH of the control samples developed normally reaching pH 5.4 within 24 hours while the pH of the samples containing antimicrobial mixtures increased slightly and stabilized at pH 5.7.

Conclusion

The synergistic activity of bacteriocin and phenolic acid was able to significantly inhibit *Salmonella* in the meat product and its efficacy was concentration dependent. A first rapid reduction of the pathogen counts as well as a sustained inhibitory effect throughout the shelf-life was achieved. However, the antimicrobial mix inhibited the starter culture and led to higher pH compared to the controls. The development of resistant starter cultures may enhance the effect of the antimicrobial combination due to the decrease of the pH during fermentation.

Notes