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SEVERAL BACTERIOCINS ENHANCE THE BACTERICIDAL EFFECT OF HIGH HYDROSTATIC PRESSURE AGAINST FOODBORNE PATHOGENS IN A MEAT MODEL

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Background

The effectiveness of high hydrostatic pressure on the destruction of cells of several foodborne pathogens in phosphate buffer and in some foods has been published (Shigehisa *et al.*, 1991, Styles *et al.*, 1991, Patterson & Kilpatrick, 1998). Complex, low-acidity food matrices, such as meat and milk, tend to protect bacteria against high pressure inactivation compared with phosphate buffer. In general, the higher the pressure and time of treatment the higher the bacterial destruction. However, several foods like meat products with a high content of proteins are not suitable to be treated at a very high pressure without altering their structure and color. For this reason it will be necessary to combine other hurdles, as for example in a four-dimensional process, including: pressure x time x temperature x antimicrobial preservatives (Kalchayanand *et al.*, 1998). Bacterial cells surviving pressurization become sublethally injured and are killed by antibacterial compounds as bacteriocins; thus viability loss of foodborne pathogens may be increased in the presence of bacteriocins and consequently the shelf life of the pressurized products extended.

Objectives

The aim of this study was to assess the effect of high hydrostatic pressure in combination with bacteriocins on the behaviour of several foodborne pathogens in a meat model system, during storage at 4ºC.

Methods

A model meat system was designed consisting of cooked ham homogenized with distilled water (1:3) distributed in plastic pouches containing bacteriocins as follows: Enterocins A,B, Sakacin K, Pediocin AcH, Nisin (Nisaplin) and a Control with no added bacteriocin. Each bacteriocin was added independently at 1280 AU/g. The titer was determined against *Listeria innocua* CTC1014 or against *Lactobacillus sake* CTC746 for Nisaplin. Each plastic pouch was inoculated to a final concentration of 10^8 CFU/g of an stationary phase culture of each selected strain. The strains tested were *Escherichia coli* CTC1007, CTC1018, CTC1023; *Salmonella* spp. CTC1003, CTC1015; *Staphylococcus aureus* CTC1008, CTC1019, CTC1021 and *Listeria monocytogenes* CTC1010, CTC1034. All strains were from meat origin.

The samples were vacuum sealed, pressurized at 400 MPa at 17ºC for 10 minutes (Alstom, Nantes, France) and stored at 4ºC for up to 61 days. At selected sampling times CFU were enumerated. Plating was done in selective media.

Results and Discussion

Limited studies have indicated that among the foodborne pathogens, some strains could be more resistant to high pressure than others. For this reason, different strains from the same species were used in this study. *E.coli* and *Salmonella* strains showed a different behaviour during the storage after pressurization. The results obtained will be useful for designing, in the future, processing parameters to ensure the safety of pressurized foods.

In general, the high pressure treatment at 400 MPa at 17ºC for 10 minutes diminished the viable counts of bacteria about 6 log cycles, except when the target strains were *S.aureus* (Tables 1&2). However, the survivors outgrew during the storage at 4ºC, except *Salmonella* (all treatments) or *E.coli* when nisin was included. When *Listeria monocytogenes* was used as a target strain, the survivors after pressurization dramatically recovered till the level of inocula (10^9 CFU/g) except when Sakacin K, Enterocins A,B or Pediocin AcH were included. The counts remained under the detection limit (2 log CFU/g) till 46 days.

Conclusion

The addition of selected bacteriocins increased the lethal effect of moderate (400 MPa) high pressure. In hydrostatic pressure-pasteurization of foods, the combination of factors in order to obtain high levels of destruction of pathogens will be the key to ensure safety of the consumers.

References

Kalchayanand, N., Sikes, A., Dunne, C. P. & Ray, B. (1998). Factors influencing death and injury of foodborne pahogens by hydrostatic pressure-pasteurization. *Food Microbiol.* 15, 207-214.

Patterson, M. F. & Kilpatrick, D. J. (1998). The combined effect of high hydrostatic pressure and mild heat on inactivation of pathogens in milk and poultry. *J. Food Protect.* 61, 432-436.

Shigehisa, T., Ohmori, T., Saito, A., Taji, S. & Hayashi, R. (1991). Effects of high hydrostatic pressure on characteristics of pork slurries and inactivaton of microorganisms associated with meat and meat products. *Int. J. Food Microbiol.* 12, 207-216.

Styles, M.F., Hoover, D.G., & Farkas, D.F. (1991). Response of *Listeria monocytogenes* and *Vibrio paraemolyticus* to high hydrostatic pressure. *J. Food Sci.* 56, 1404-1407.

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TABLE 1. Behaviour of bacteria in a meat model after pressurization (400 MPa 10min 17ºC) with bacteriocins and storage at 4ºC

	0	1 day	4 days	7 days	11 days	18 days	26 days	46 days	61 days
***Escherichia coli* CTC1018**									
Control	7.59	4.32	4.62	4.94	5.55	4.85	5.72	5.42	5.80
Ent A, B	7.59	3.48	4.57	5.47	5.07	4.91	5.41	7.47	6.94
Sak K	7.59	4.50	4.91	5.07	4.85	4.77	5.12	5.80	5.47
Ped AcH	7.59	3.42	4.13	4.56	4.29	4.52	5.93	8.73	8.67
Nisin	7.59	1.99	1.99	2.24	2.30	2.50	2.39	2.77	3.98
***Escherichia coli* CTC 1007**									
Control	7.46	2.00	3.75	3.95	4.39	3.74	3.85	3.54	2.30
Ent A, B	7.46	2.00	3.45	3.33	3.86	3.35	6.62	6.99	7.04
Sak K	7.46	3.36	3.45	3.78	4.28	3.72	4.13	5.79	5.33
Ped AcH	7.46	2.58	3.37	3.39	3.30	3.54	3.67	3.00	2.59
Nisin	7.46	1.99	2.00	1.99	1.99	1.99	1.99	1.99	1.99
***Escherichia coli* CTC1023**									
Control	8.16	3.68	3.54	4.40	5.02	3.69	3.59	4.60	3.50
Ent A, B	8.16	2.30	3.19	4.16	4.53	3.60	4.23	4.16	3.25
Sak K	8.16	4.34	3.64	4.73	5.04	4.38	4.48	5.24	4.46
Ped AcH	8.16	4.20	3.73	4.36	4.12	3.91	4.29	4.67	4.04
Nisin	8.16	2.00	1.99	1.99	1.99	1.99	1.99	1.99	1.99
***Salmonella* CTC1003**									
Control	7.62	1.99	2.35	2.52	3.40	3.20	3.34	2.42	2.24
Ent A, B	7.62	1.99	2.45	2.63	2.99	2.89	2.69	2.30	1.99
Sak K	7.62	1.99	1.99	2.00	2.15	2.30	2.15	2.00	1.99
Ped AcH	7.62	1.99	1.99	1.99	2.00	2.00	2.15	2.00	2.00
Nisin	7.62	1.99	1.99	1.99	1.99	2.00	1.99	1.99	1.99
***Salmonella* CTC1015**									
Control	7.49	1.99	1.99	1.99	1.99	1.99	1.99	1.99	1.99
Ent A, B	7.49	1.99	1.99	1.99	1.99	1.99	1.99	1.99	1.99
Sak K	7.49	1.99	1.99	1.99	1.99	1.99	1.99	1.99	1.99
Ped AcH	7.49	1.99	1.99	1.99	1.99	1.99	1.99	1.99	1.99
Nisin	7.49	1.99	1.99	1.99	1.99	1.99	1.99	1.99	1.99

Values are expressed in $\log_{10}$ CFU/g. Values are the average of duplicate.

TABLE 2. Behaviour of bacteria in a meat model after pressurization (400 MPa 10min 17ºC) with bacteriocins and storage at 4ºC

	0	1 day	4 days	7 days	11 days	18 days	26 days	46 days	61 days
***Staphylococcus aureus* CTC1008**									
Control	8.51	7.93	8.21	8.18	7.90	7.98	7.92	7.19	6.51
Ent A, B	8.51	8.65	8.36	8.29	8.29	8.07	8.00	7.65	6.46
Sak K	8.51	8.46	8.42	8.24	8.24	8.01	8.07	7.68	6.86
Ped AcH	8.51	8.35	8.23	8.45	8.16	8.16	7.82	7.51	6.64
Nisin	8.51	8.21	7.63	7.79	8.33	7.39	7.30	6.90	6.08
***Staphylococcus aureus* CTC1019**									
Control	8.13	7.77	8.25	8.34	8.09	8.15	8.16	7.93	7.47
Ent A, B	8.13	7.88	8.16	8.15	8.15	8.03	8.00	7.57	7.05
Sak K	8.13	8.11	8.31	8.35	8.31	8.23	8.11	7.93	7.49
Ped AcH	8.13	8.02	8.20	8.39	8.18	8.24	8.16	7.59	7.33
Nisin	8.13	8.05	7.98	8.19	8.00	7.66	7.46	7.01	6.66
***Staphylococcus aureus* CTC1021**									
Control	8.45	8.19	8.06	8.44	8.02	8.18	8.00	7.91	7.22
Ent A, B	8.45	8.18	8.39	8.54	8.37	8.37	8.18	7.86	7.48
Sak K	8.45	8.45	8.22	8.44	8.30	8.40	8.25	7.93	7.32
Ped AcH	8.45	8.08	8.34	8.59	8.41	8.36	8.26	7.95	7.40
Nisin	8.45	8.40	8.08	8.33	8.15	8.19	7.99	7.21	7.11
***Listeria monocytogenes* CTC1010**									
Control	8.44	2.00	2.63	3.05	3.66	5.13	7.47	8.39	9.22
Ent A, B	8.44	1.99	1.99	1.99	1.99	1.99	1.99	1.99	2.35
Sak K	8.44	1.99	1.99	1.99	1.99	1.99	1.99	1.99	3.59
Ped AcH	8.44	1.99	1.99	1.99	1.99	1.99	1.99	1.99	1.99
Nisin	8.44	1.99	1.99	1.99	2.00	3.30	3.30	7.79	9.36
***Listeria monocytogenes* CTC1034**									
Control	8.27	1.99	1.99	2.43	3.70	6.14	8.67	9.33	9.43
Ent A, B	8.27	1.99	1.99	1.99	1.99	1.99	1.99	1.99	1.99
Sak K	8.27	1.99	1.99	1.99	1.99	1.99	1.99	2.00	1.99
Ped AcH	8.27	1.99	1.99	1.99	1.99	1.99	1.99	1.99	3.33
Nisin	8.27	1.99	1.99	2.15	2.15	3.62	6.32	8.88	9.53

Values are expressed in $\log_{10}$ CFU/g. Values are the average of duplicate.