ENHANCEMENT OF SAFETY OF PROCESSED MEAT BY HYDROSTATIC PRESSURE IN COMBINATION WITH TEMPERATURE AND BACTERIOCIN

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Background

High hydrostatic pressure (HHP) can both kill and injure microbial cells (Kalchayanand, et al., 1994) and is being studied as a novel means to enhance microbial safety and shelf-life of foods (Hoover, 1993). Destruction of about 8 log cycles of some pathogenic bacteria in phosphate buffer at 22°C may need pressure of 700 MPa for 20 min (Patterson et al., 1995). We showed that ≥8 log cycles viability loss of foodborne pathogenic and spoilage bacterial cells could be accomplished at 345 MPa in 5 min by pressurizing the cells at 50°C in the presence of a bacteriocin, pediocin AcH (Kalchayanand, 1998 a,b). The enhanced viability loss was attributed to the phase transition of cell membrane lipids and greater sublethal injury of cells (Ray, 1993; Kalchayanand et al., 1992).

Objectives

In this study we determined the effectiveness of combination of moderate hydrostatic pressure and temperature along with pediocin AcH to: (a) reduce a high population of pathogenic bacteria in processed meat products, and (b) enhance safety of processed meat products during extended storage at 25°C.

Methods

Roast beef, Cotto salami and summer sausage were used. Roast beef was prepared by cooking to 71.1°C and immediately chilling to 4°C. Cotto salami was formulated with beef trim, beef fat, salt, cure, erythorbate, trisodium phosphate, seasoning and with or without pediocin AcH (3,000 AU/g). After cooking at 68.3°C the product was chilled to 4°C. Summer sausage was formulated similar to Cotto salami but contained 1% dextrose. Also, cells of pediocin AcH producing strain was added to the ingredients. Following incubation at 36.1°C until the pH dropped to 5.0 the sausages were cooked at 62.8°C and cooled to 4°C. All three products were then sliced aseptically to about 84 g portions and put in low oxygen permeable plastic bags (1 portion/bag).

The pathogens *Staphylococcus aureus* 582, *Listeria monocytogenes* Scott A, *Salmonella typhimurium* ATCC 14028 and *Escherichia coli* 0157:H7 #932 were grown at 37°C to stationary phase in tryptic soy broth and inoculated separately to the meat products at high level (10^7 to 10^8/g) or low level (10^3 to 10^4/g). When required, pediocin AcH was also applied at 3,000 AU/g. Two propriotory bacteriocin based biopreservatives, BP1 and BP1x, were also used (Michigan Biotechnology Institute, MI). The bags were vacuum-sealed and pressurized at 345 MPa and 50°C for 5 min and cooled to 4°C immediately. For the products inoculated with high levels of pathogens, surviving and injured cells were enumerated immediately in tryptic soy agar (TSA, nonselective) and selective agar medium plates. Products inoculated with low levels of pathogens were stored at 25°C and tested at selected intervals for the presence of pathogens. Duplicate samples were used at each test period.

Results and Discussion

Roast beef samples were inoculated with $1.7x10^7$ to $2.2x10^8$ cfu/g of the four pathogens (Table 1). Pressurization at 345 MPa and 50°C for 5 min reduced viability ranging between 4 to 7 log cycles; *E. coli* showed the most sensitivity. Some survivors failed to form colonies in the selective media due to injury (Kalchayanand et al., 1994). The cfu of *Sta. aureus* and *Lis. monocytogenes* were lower in the presence of pediocin AcH, BP1 and BP1x, due to their sensitivity to bacteriocins and BP's seemed to be more effective. *Sal. typhimurium* had less sensitivity to bacteriocin preparations as their bactericidal effect to injured Gram-negative bacteria is greater during storage (Kalchayanand et al., 1992; Ray, 1993). In Cotto salami, pressurization resulted in 5 to 7 log cycles viability loss and the survivors had injured cells. Pressurization with bacteriocin preparations killed more cells. Pressurization of the summer sausage (pH 5) produced higher viability loss than the other two products.

Storage studies with pressurized roast beef and summer sausage were conducted with low levels of pathogens (Table 2). In the roast beef with initial $3x10^3$ to $1.5x10^4$ cfu/g, pressurization alone at 345 MPa and 50°C for 5 min killed all the cells of the three pathogens as no survivors were detected during 84 d storage at 25°C. Only *Sta. aureus*, although not detected initially, were detected after 1 week, even in samples containing bacteriocin preparations. In summer sausage four pathogens were not detected following pressurization and up to 84 d storage at 25°C.

Conclusion

Low heat processed meat products can be contaminated by pathogenic and spoilage bacteria which can multiply during storage. Moderate hydrostatic pressure, moderate temperature and suitable biopreservative could be used to overcome this problem (data from spoilage bacteria not presented).

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Pertinent Literature

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Table 1. Viability loss and injury of foodborne pathogens in processed meat following hydrostatic pressure treatment.

Pathogens/Treatment[a]	cfu/g of Roast beef on TSA	Roast beef on SA[b]	Cotto salami on TSA	Cotto salami on SA	Summer sausage on TSA	Summer sausage on SA
***E. coli* 0157:H7**						
Control	$1.7x10^7$	$1.3x10^7$	$1.2x10^8$	$4.0x10^7$	$2.8x10^2$	$1.2x10^7$
HP	<10	<10	$5x10^1$	$1.0x10^1$	<10	<10
HP+P	<10	<10	$4x10^1$	<10	<10	<10
HP+BP1	<10	<10	$1x10^1$	<10	<10	<10
HP+BP1x	<10	<10	$1x10^1$	<10	<10	<10
Sal. typhimurium						
Control	$2.3x10^7$	$4.0x10^6$	$1.2x10^8$	$5.0x10^7$	$2.4x10^7$	$4.0x10^6$
HP	$4.0x10^3$	<10	$1.0X10^3$	<10	<10	<10
HP+P	$3.0x10^3$	<10	$2.0x10^1$	<10	<10	<10
HP+BP1	$2.8x10^3$	<10	$1.0x10^1$	<10	<10	<10
HP+BP1x	$2.4x10^3$	<10	$1.0x10^1$	<10	<10	<10
Lis. monocytogenes						
Control	$2.2x10^8$	$1.6x10^8$	$1.5x10^8$	$9.0x10^7$	$1.8x10^8$	$1.4x10^8$
HP	$2.8x10^3$	$7.0x10^2$	$1.0x10^3$	$9.0x10^1$	<10	<10
HP+P	$8.0x10^2$	<10	$6.0x10^2$	<10	<10	<10
HP+BP1	<10	<10	$1.0x10^1$	<10	<10	<10
HP+BP1x	<10	<10	<10	<10	<10	<10
Sta. aureus						
Control	$6.0x10^3$	$2.6x10^7$	$1.5x10^8$	$1.1x10^8$	$4.0x10^7$	$3.0x10^7$
HP	$2.5x10^3$	$2.2x10^3$	$4.0x10^2$	$4.0x10^1$	<10	<10
HP+P	$6.0x10^2$	$4.0x10^1$	$2.0x10^2$	$1.0x10^1$	<10	<10
HP+BP1	$3.0x10^2$	<10	$4.0x10^1$	<10	<10	<10
HP+BP1x	$3.0x10^2$	<10	<10	<10	<10	<10

[a]Treatment: HP, hydrostatic pressure at 345 MPa for 5 min at 50°C; P, pediocin AcH; BP1 and BP1x are biopreservatives.
[b]Selective agar media: Violet red bile agar for *Escherichia*, xylose-lysine deoxycholate agar for *Salmonella*, moxalactum agar for *Listeria* and 10% NaCl tryptic soy agar for *Staphylococcus*. Tryptic soy agar (TSA) was nonselective agar. <10 indicates no cfu in 0.4 g product.

Table 2. Enumeration of foodborne pathogens following hydrostatic pressurization and storage at 25°C.

Pathogens/Treatment[a]	cfu/g on SA[b] Roast beef after 0 d	Roast beef after 7d	Roast beef after 84 d	Summer sausage after 0 d	Summer sausage after 7d	Summer sausage after 84 d
***E. coli* 0157:H7**						
Control	$5.0x10^3$	$2.0x10^{10}$	NT	$9.0x10^2$	$4.0x10^1$	<10
HP, HP+P, HP+BP1, HP+BP1x	<10	<10	<10	<10	<10	<10
Sal. typhimurium						
Control	$1.5x10^4$	$4.0x10^{10}$	NT	$7.0x10^2$	$2.0x10^2$	<10
HP, HP+P, HP+BP1, HP+BP1x	<10	<10	<10	<10	<10	<10
Lis. monocytogenes						
Control	$6.0x10^3$	$1.3x10^{10}$	NT	$4.0x10^3$	$1.0x10^1$	<10
HP, HP+P, HP+BP1, HP+BP1x	<10	<10	<10	<10	<10	<10
Sta. aureus						
Control	$3.0x10^3$	$4.0x10^8$	NT	$3.0x10^3$	$4.0x10^2$	<10
HP	<10	$1.0x10^3$	NT	<10	<10	<10
HP+P	<10	$1.0x10^2$	NT	<10	<10	<10
HP+BP1	<10	$1.0x10^2$	NT	<10	<10	<10
HP+BP1x	<10	$1.0x10^2$	NT	<10	<10	<10

[a]Treatment: HP, hydrostatic pressure at 345 MPa for 5 min at 50°C; P, pediocin AcH; BP1 and BP1x are biopreservatives.
[b]Selective agar media: Violet red bile agar for *Escherichia*, xylose-lysine deoxycholate agar for *Salmonella*, moxalachum agar for *Listeria* and 10% NaCl tryptic soy agar for *Staphylococcus*. Tryptic soy agar (TSA) was nonselective agar. <10 indicates no cfu in 0.4 g product.
[c]Day of enumeration after storage at 25°C.
[d]<10 cfu/0.4 g product following all four treatments.