COMPLETE DESTRUCTION OF *LISTERIA MONOCYTOGENES* IN HOT DOGS BY HYDROSTATIC PRESSURE AND BACTERIOCIN-BASED BIOPRESERVATIVES

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Background

Ready-to-eat (RTE) low-heat processed meat products, following heat treatment, are handled extensively prior to final packaging. During this time, the products could be contaminated with pathogenic and spoilage microorganisms. The products are vacuum-packaged and refrigerated to achieve the expected shelf-life of 8 to 12 weeks. Psychrotrophic facultative anaerobic and anaerobic pathogenic and spoilage bacteria can multiply and reduce the shelf-life and safety of the products. Foodborne disease outbreaks and deaths were reported in the United States from RTE products contaminated with psychrotrophic *Listeria monocytogenes*. There is an interest in developing intervention methods to eliminate the presence of *Lis. monocytogenes* in RTE products. High hydrostatic pressure (HP) could be a possible method. It was reported that HP destroys *Lis. monocytogenes*, in laboratory media and food and, its bactericidal efficiency was enhanced by combining with bacteriocins (Kalchayanand et all., 1998 a,b,c; Alpas et al., 1999; Patterson, et al., 1995; Styles, et al., 1991).

Objectives

The objective of this study is to determine the effectiveness of four parameters, moderate hydrostatic pressure, moderate pressurization temperature for a short time along with bacteriocin-based biopreservative in the complete destruction of pathogenic *Lis. monocytogenes* strain present in packages of hot dogs at a level of about 4 log cycles/g of product.

Methods

Three *Lis. monocytogenes* strains from our culture collection were grown in tryptic soy broth supplemented with 0.6% yeast extract (TSBY broth) at 37°C to late exponential phase, diluted with 10% peptone solution to about 10^8 to 10^9 cfu/ml, 2 ml of each were transferred into 2 ml sterile vials which were then vacuum-sealed in plastic bags. The vials were pressurized at 207, 276, or 345 MPa for 5 or 10 min at 25°, or 50°C. The vials were cooled to 4°C and used for cfu enumeration by pour plating in TSBY-agar (TSBY broth + 1.5% agar) and incubated at 37°C for 2 d (Kalchayanand et al., 1998a,b,c). Viability loss was determined by comparing the cfu of the control and pressurized cell suspensions. To determine differences in sensitivity of *Lis. monocytogenes* strains to 345 MPa for 5 min at 25°C, seven strains were used. The strains were grown, pressurized, and enumerated for survivors as before to determine relative sensitivity to 345 MPa. To determine the viability loss of *Lis. monocytogenes* strains by pressurization at 345 MPa for 5 min at 50°C in the absence and presence of two bacteriocin-based biopreservatives (BP and BPx), four strains were grown, mixed and pressurized with and without BP or BPx as before. BP was prepared by mixing the two bacteriocins, pediocin AcH and nisin A (3:7 ratio), BPx was prepared by including lysozyme to BP. The final concentrations of the bacteriocins and lysozyme were 5,000 AU and 100 µg/ml. The survivors were enumerated and viability loss for each treatment was determined. In product inoculation studies, commercial hot dogs were dipped in boiling water for 2 min and cooled to 4°C. One hot dog (about 35 g) was put in a sterile plastic bag inoculated with cell suspension of four strain mixture (to give about 10^4 cells/g along with or without 1 ml BP or BPx). The hot dog packages were pressurized at 345 MPa for 5 min at 50°C using four packages for each treatment. The bags were stored at 4°C and examined for survivors and growth after 1 d and 7 d of storage. Following opening each bag, 35 ml of UVM broth was added and 1 ml and 0.1 ml portions in duplicate were pour plated with 5 ml TSY-agar/plate. After 2 h about 10 ml melted MOX-agar was poured on each plate. Following incubation for 2 d, black colonies were enumerated. The remaining UVM broth were incubated at 37°C for 24 h and a loopful of broth was streaked on a MOX-agar plate. Following 2 d at 35°C, the plates were examined for characteristic growth.

Results and Discussion

These relative viability loss of *Lis. monocytogenes* strains following pressurization at 207, 276 or 345 MPa for 5 or 10 min at 25° or 50°C is presented in Table 1. In the three strains viability loss increased with the increase in pressure and pressurization time and temperature. However, proportionately more cells were killed by increasing the pressure to 345 MPa from 276 MPa than by increasing from 207 MPa to 276 MPa. Similarly, at 50°C proportionately more cells were killed than at 25°C; however, pressurization at 5 min or 10 min did not proportionately increase the viability loss. A combination of moderate pressure (namely 345 MPa) and moderately high temperature (namely 50°C) can be effective in reducing viability of *Lis. monocytogenes* strains in 1% peptone suspension by 8 log cycles. Similar observations were reported by others (Alpas et al., 1999; Kalchayanand et al., 1998a,b; Patterson and Kilpatrick, 1998; Patterson et al., 1995). Six *Lis. monocytogenes* strains were examined for relative sensitivity to pressurization at 345 MPa at 25°C for 5 min. Results in Table 2 showed the viability loss ranged between 1.2 and 1.7 log cycles. To determine viability following pressurization at 50°C, four strains (CA, OB90684, OB90339, and OB90759) were grown separately, mixed in equal cell density and used. Results in Table 3 showed that at 50°C the viability loss due to pressurization of the mixed population was 5.8 log cycles. However, when the cells were pressurized at 50°C in the presence of the two bacteriocin-based biopreservatives, the viability loss was over 8.0 log cycles. Similar observations were made for several pathogenic and spoilage bacteria (Kalchayanand et al., 1998a,b,c). The effectiveness of 5 min pressurization at 345 MPa and 50°C on the destruction of about 4 log cycles (/g) of four-strains mixture of *Lis. monocytogenes* in hot dogs in the presence and absence of BP and BPx is presented in Table 4. In the control samples the cfu slightly decreased after 1 d but showed increase due to growth after 7 d at 4°C. The pressurized samples had 1.8×10^1 cfu/g showing a reduction of 95% (or 1.8 log cycles). This is much less than the 5.8 log cycle reduction obtained in broth (Table 3). This could be due to both the effect of the product and the ineffectiveness of heating the product to 50°C. This is currently being studied. However, in the presence of either BP or BPx all samples tested following storage at 4°C after 1 d and 7 d of pressurization no *Lis. monocytogenes* cfu was enumerated. When the hot dog samples with broth were incubated at 37°C for 24 h, no *Lis. monocytogenes* growth was observed in any of the samples with BP or BPx. This suggests that the destruction of *Lis. monocytogenes* in processed meat by hydrostatic pressure could be enhanced in the presence of a bacteriocin-based biopreservative.

Conclusion

Lis. monocytogenes can contaminate properly processed ready-to-eat processed meat products as postheat contaminants and grow during refrigerated storage and temperature abuse. The health hazard from this pathogen can be controlled by subjecting the packaged products, containing a bacteriocin-based biopreservative, to moderately high hydrostatic pressure preferably at a moderate temperature.

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Pertinent Literature

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Table 1. Viability loss of *Listeria monocytogenes* strains as influenced by hydrostatic pressure and pressurization temperature and time.

		Viability loss (log_{10} cfu/ml)[a]					
		207 MPa		276 MPa		345 MPa	
Strains	0°C	5 min	10 min	5 min	10 min	5 min	10 min
CA	25	0.5	0.7	0.6	0.8	0.9	2.4
	50	3.0	3.1	6.5	6.8	≥8.0	≥8.0
Scott A	25	0.7	0.8	0.7	0.8	3.0	5.3
	50	3.0	3.8	6.7	7.0	≥9.0	≥9.0
OH_2	25	0.6	0.8	0.7	0.9	2.6	3.1
	50	3.1	3.3	8.1	8.1	≥8.0	≥8.0

[a]Cells (8 to 9 log cycles) were suspended in 1% peptone broth and pressurized. Results are average of two samples. D values (min to reduce population by 1 log cycle) at 345 MPa at 50°C, in 5 min was ≤0.6.

Table 2. Differences in viability loss among *Lis. monocytogenes* strains to pressurization at 345 MPa for 5 min at 25°C.

		log_{10} cfu/ml	
Strains[a]	Sources	Before pressure	Viability loss[b]
CA	Soft cheese	7.9 ± 0.2	1.3 ± 0.4[c]
OB90684	Ham	7.7 ± 0.4	1.2 ± 0.4[c]
OB90339	Cooked chicken	7.9 ± 0.2	1.4 ± 0.2[c]
OB90508	Ham	7.9 ± 0.1	1.5 ± 0.3
OB90303	Roasted turkey	8.0 ± 0.3	1.6 ± 0.2
OB90477	Ham	7.9 ± 0.1	1.6 ± 0.2
OB90759	Beef frank	7.8 ± 0.2	1.7 ± 0.7[c]

[a]All strains, except CA, were from USDA laboratory.
[b]Cells were suspended in 1% peptone solution.
[c]The cell suspensions of four strains were used to prepare composite in other studies.

Table 3. Effect of pressurization at 345 MPa for 5 min at 50°C on viability loss of 4 strains-mixture of *Lis. monocytogenes*.

Treatment	log_{10} cfu/ml	Viability loss
Control	8.2 ± 0.1	---
Hyd. pressure	2.4 ± 0.9	5.8 (1.2 to 1.7 at 25°C)
Hyd. pressure + BP	<1.0[b]	≥8.2
Hyd. pressure + BPx	<1.0	≥8.2

[a]Cell suspensions contained a biopreservative (pediocin AcH + nisin in 1:1) at 5,000 AU/ml (BP) and BP + lysozyme (100 µg/ml) BPx.
[b]No cfu was detected in 1 ml cell suspension from each sample.
[c]Viability loss due to BP and 50°C for 10 min ≃ 1 log cycle.

Table 4. Effectiveness of destruction of pressurization and biopreservatives on destruction 4 strains-mixture of *Lis. monocytogenes* in hot dogs.

	log_{10} cfu/g at 4°C			No. positive/No. tested	
Treatment[a]	0 d	1 d	7 d	1 d	7 d
Control	4.2×10^2	3.6×10^2	5.2×10^4	4/4	4/4
Hyd. pressure	NT	1.8×10^1	4.8×10^2	4/4	4/4
Hyd. pressure + BP	NT	$<10^0$	$<10^0$	0/4	0/4
Hyd. pressure + BPx	NT	$<10^0$	$<10^0$	0/4	0/4

[a]Inoculated hot dogs were pressurized at 345 MPa at 50°C for 5 min in plastic bags.