

Interaction of Hydrostatic Pressure, Time and Temperature of Pressurization and Pediocin AcH on Inactivation of Foodborne Bacteria

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MS 97-94: Received 5 May 1997/Accepted 14 September 1997

ABSTRACT

High hydrostatic pressure, because it can kill microorganisms, is being investigated for potential use as a nonthermal food preservation method. The objective of this study was to determine the hydrostatic pressurization parameters, pressure, time, and temperature, and a bacteriocin that in combination would destroy 7 to 8 log cycles of pathogenic and spoilage bacterial populations. We suspended cells of *Staphylococcus aureus*, *Listeria monocytogenes*, *Salmonella typhimurium*, *Escherichia coli* O157:H7, *Lactobacillus sake*, *Leuconostoc mesenteroides*, *Serratia liquefaciens*, and *Pseudomonas fluorescens* in peptone solution and exposed them to the combination of treatments. The combined parameters used were hydrostatic pressure (138 to 345 MPa), time (5 to 15 min), temperature (25 to 50°C), and pediocin AcH (3,000 AU/ml, final concentration). In general, cell death increased as the pressure, time, or temperature increased; however, the cells developed proportionately greater sensitivity as the pressure increased to 276 MPa and higher and the temperature increased above 35°C. Pressurization for longer than 5 min, especially at lower pressure and temperature ranges, had very little added benefit. Among the four gram-negative species, *E. coli* O157:H7 was the most resistant to pressurization while among the four gram-positive species, *L. sake* and *L. mesenteroides* had greater resistance. The death rate at high pressure (345 MPa) and high temperature (50°C) in combination followed first-order kinetics; at a lower pressure and temperature combination it showed a late tailing effect. Estimated *D* value data indicated that even at 345 MPa and 50°C an 8-log-cycle viability loss could not be achieved within 5 min for all eight species. However, when pediocin AcH was included during pressurization this loss was achieved.

The potential of high hydrostatic pressure, because it can kill microorganisms without altering the flavor and nutrient content of a food, is being investigated as a novel method of food preservation. In addition, hydrostatic pressure is energy efficient, with a minimal sanitation problem, and the antimicrobial effect is isostatic and instantaneous (6, 7, 11). However, the process has several disadvantages. Although vegetative bacteria and fungi are destroyed at lower pressures, the destruction of bacterial spores requires very high pressure. At very high pressures the process may not be economical for commercial use due to the high cost of equipment and increased metal fatigue (4, 7, 13). In addition, the normal texture and color of some foods may be adversely altered at high pressure (3, 8, 19). Because of these problems, it has been suggested that commercial sterilization at high pressure and pressure pasteurization at low pressure (as in thermal processing) should be investigated separately (9, 17).

The effectiveness of hydrostatic pressure pasteurization on the destruction of several foodborne pathogens, namely *Salmonella* spp., *Escherichia coli* O157:H7, *Campylobacter jejuni*, *Vibrio parahaemolyticus*, *Listeria monocytogenes*, and *Staphylococcus aureus* has been reported (9, 10, 14, 15, 18, 20). Few investigators have studied food spoilage

bacteria. With pathogens, the cells were usually suspended in a phosphate buffer or in some foods and pressurized between 140 to over 700 MPa for 5 to 80 min at 20 to 25°C (14, 15, 18, 20). Studies have revealed that (i) cell viability loss increased with increase in pressure and time; (ii) up to 200 MPa, even after 30 min, cell death was ≤ 1 log cycle; (iii) the death rate did not follow first-order kinetics, but above 275 MPa the death rate had an initial exponential phase followed by a tailing effect; (iv) gram-negative bacteria showed more sensitivity than gram-positive bacteria, and in both groups the species and strains differed in sensitivity to pressure; and (v) viability loss is lower in a food system than in phosphate buffer. For *E. coli* O157:H7 and *S. aureus*, a pressure as high as 700 MPa at 20°C for 15 min was required for a 5-log-cycle reduction (15). *D* values for *L. monocytogenes* CA and *S. typhimurium* ATCC 7136 at 345 MPa and 20°C in phosphate buffer were 6.7 and 7.4 min, respectively (14, 20). Although destruction of bacterial pathogens by hydrostatic pressure is readily attainable, careful kinetic studies are necessary to ensure a desired level of cell destruction and food safety (12). This information is important for commercial application of pressure pasteurization of food much below 700 MPa for a shorter time (probably not exceeding 10 min) to produce a cell destruction of about 7 to 8 log cycles. Similar information will also be important for spoilage bacteria to ensure a long shelf life for pressure-pasteurized foods.

Recent studies have indicated that bacterial cells are the least sensitive to hydrostatic pressure at 20 to 25°C but sensitivity increases above 30°C due to a phase transition in the membrane (12). Pressurization also inflicts sublethal injury in both gram-positive and gram-negative bacterial cells and makes them susceptible to antibacterial compounds, such as bacteriocins and lysozyme (5, 9, 10). We hypothesized that it would be possible to obtain a desired level of bacterial cell destruction within a short time at a moderate hydrostatic pressure by also applying a moderate temperature increase and suitable antibacterial compounds. We studied the combined effect of pressure, time, and temperature and a bacteriocin on the rate of viability loss of four pathogenic and four spoilage bacteria and determined the minimum conditions of these four parameters that can reduce viability of all eight species by about 7 to 8 log cycles.

MATERIALS AND METHODS

Bacterial species. Eight bacteria were studied: *Staphylococcus aureus* 582 (from the U.S. Food and Drug Administration Food Microbiology Laboratory, Washington, D.C.), *Listeria monocytogenes* Scott A, *Escherichia coli* O157:H7-932 (both from M. Doyle, University of Georgia, Griffin), *Salmonella typhimurium* ATCC 14028 (U.S. Army Research, Natick, MA), *Leuconostoc mesenteroides* Ly, *Lactobacillus sake* FM1, *Pseudomonas fluore-*

scens FM1, and *Serratia liquefaciens* FM1 (all four from our stock collection). Except for *S. typhimurium*, all pathogens were isolated from foods implicated in foodborne diseases. The spoilage bacteria, consisting of two gram-positive (*L. mesenteroides* and *L. sake*) and two gram-negative (*P. fluorescens* and *S. liquefaciens*) species, were isolated from spoiled vacuum-packaged refrigerated processed meat products in our laboratory. All strains were maintained as frozen stock. For this study all four pathogens and *P. fluorescens* and *S. liquefaciens* were grown in tryptic soy broth (Difco Laboratories, Detroit, MI) supplemented with 0.6% yeast extract (TSY broth). *L. mesenteroides* and *L. sake* were grown in tryptone-glucose-yeast extract (TGE) broth (1). The cultures were maintained at room temperature (22 to 25°C) and transferred twice every week.

Preparation of cell suspensions for pressurization. The four pathogens and *S. liquefaciens* and *P. fluorescens* were grown in TSY broth at 37°C; *L. sake* and *L. mesenteroides* were grown in TGE broth at 30°C for 16 to 18 h. The cells were harvested by centrifugation at 7,700 × g for 10 min at 10°C, washed with sterile 0.1% peptone solution and resuspended in the same solution to a population of about 10⁸ to 10⁹ CFU/ml. The cell suspensions were dispensed in 2-ml portions in sterile plastic cryovials (2 ml capacity; Simport Plastic, Beloell, Canada). The vials were individually vacuum-sealed in Whirl Pak plastic bags (Nasco, Atkinson, WI) and pressurized. The controls were unpressurized vials containing the cell suspensions. The vials were kept at 4°C during handling before and after pressurization that did not exceed 1 h.

TABLE 1. Survivors of four foodborne pathogens following combined treatment of hydrostatic pressure, time, and temperature

Bacterial species	Temperature (°C)	Mean log ₁₀ CFU/ml (n = 2) following pressurization at											
		138 MPa for			207 MPa for			276 MPa for			345 MPa for		
		5	10 min	15	5	10 min	15	5	10 min	15	5	10 min	15
<i>Staphylococcus aureus</i> 582	25	8.7 (8.7) ^a	8.8	8.7	7.7 (8.8)	7.0	6.6	5.5 (8.7)	4.8	4.1	1.0 (8.8)	0.7	ND ^b
	35	8.1	7.9	7.8	6.9	6.8	6.0	3.9	3.0	2.2	ND ^b	ND	ND
	45	7.0	6.0	5.2	3.0	2.7	2.6	1.3	1.0	1.0	ND	ND	ND
	50 ^c	4.2	4.2	4.0	2.8	2.4	2.0	1.3	ND	ND	ND	ND	ND
<i>Listeria monocytogenes</i> Scott A	25	8.9 (9.0)	8.1	7.9	8.2 (8.9)	8.1	8.0	7.3 (8.0)	7.2	7.1	6.0 (9.0)	3.7	2.9
	35	8.0	8.0	8.0	8.1	8.0	7.8	7.1	6.1	5.6	1.7	ND	ND
	45	7.2	7.1	7.0	5.9	5.1	4.1	3.0	2.8	1.6	ND	ND	ND
	50	4.4	4.4	3.7	2.0	1.6	1.3	1.3	1.0	ND	ND	ND	ND
<i>Salmonella typhimurium</i> ATCC 14028	25	7.3 (7.5)	7.3	7.3	6.8 (7.5)	6.8	6.6	4.6 (7.8)	3.4	2.3	ND (8.0)	ND	ND
	35	7.3	7.0	7.0	6.8	6.5	6.3	1.5	ND	ND	ND	ND	ND
	45	6.6	6.3	6.3	6.0	5.8	5.3	ND	ND	ND	ND	ND	ND
	50	3.0	2.7	2.6	ND	ND	ND	ND	ND	ND	ND	ND	ND
<i>Escherichia coli</i> O157:H7 #932	25	6.5 (6.7)	6.5	6.5	6.5 (6.7)	6.0	5.6	4.6 (7.5)	4.5	4.1	2.5 (7.5)	1.3	ND
	35	6.5	6.5	6.3	6.3	6.3	5.8	3.5	3.5	3.0	2.3	1.0	ND
	45	6.5	5.9	5.9	2.8	1.9	1.5	ND	ND	ND	ND	ND	ND
	50	5.3	4.2	4.1	1.0	ND	ND	ND	ND	ND	ND	ND	ND

^a Values in parentheses represent unpressurized control samples for a data set of time and temperature at one pressure.

^b ND, no CFU detected in total of 0.4 ml from duplicate samples. Each value is mean of duplicate samples.

^c Heating at 50°C for 15 min in 0.1% peptone solution reduced viability by 0.7 to 2.9 log cycles of the four pathogens; *E. coli* O157:H7 was the least sensitive with a viability loss of 0.7 log cycle. Viability loss of bacterial cells by the combined treatment of pressure and temperature is not additive of individual treatments.

Bacteriocin treatment. The pediocin AcH producer, *Pediococcus acidilactici* LB 42-923, was grown in TGE broth in a fermentor (Biostat M, B. Braun, Allentown, PA) at 37°C for 18 h under conditions for optimum production of the bacteriocin (1, 22). Pediocin AcH was partially purified from the culture broth by the method of pH-dependent adsorption and desorption of the molecules on and from the cell surface of the producer strain (21). The freeze-dried preparation was assayed for activity units (AU)/mg of powder against a lawn of *Lactobacillus plantarum* NCD0955 (16) and stored at -20°C. For this study, the required amount of the freeze-dried powder was dissolved in sterile deionized water, assayed for AU/ml, and added to the cell suspensions at a final level of 3,000 AU/ml before dispensing cells into the cryovials prior to pressurization.

Hydrostatic pressurization of cell suspensions. A hydrostatic pressurization unit (Engineered Pressure Systems, Andover, MA) capable of operating up to 690 MPa between 25 to 95°C was used. The pressure chamber (6 cm i.d. by 21 cm length) was filled with a mixture of deionized water and 5% oil (Mobile Hydrosol 78). The liquid can be heated to a desired temperature prior to pressurization by an electric heating system around the chamber. The rate of increase in pressure level was about 140 MPa/min and the pressure come-down time was about 2 min. Pressurization time reported in this study did not include the come-up and come-down times. The pressure level and time and temperature of pressurization were set by an automatic controlled device which also recorded all the parameters during the pressurization cycle.

The temperature of the liquid inside the pressure chamber was set between 25 to 50°C as required in a specific study. The cryovials, in plastic bags, containing the cell suspension (with or without pediocin AcH) were placed in a wire basket and submerged in the liquid in the pressure chamber for 2 min to allow temperature equilibration of the cell suspensions. Duplicate vials were used for each treatment. After closing the chamber the cells were exposed to a desired level of pressure by time by temperature combinations which varied between 138 and 345 MPa, 5 and 15 min and 25 and 50°C. Immediately after pressurization the vials were taken out, kept at 4°C and the cell suspensions (both control and treated) were enumerated for viable cells (10).

Enumeration of viable cells. The cell suspension in each vial was serially diluted in 0.1% sterile peptone solution and 0.1-ml portions were surface plated in duplicate. Tryptic soy agar (Difco) supplemented with 0.6% yeast extract (TSY agar) was used to enumerate the pathogens and *P. fluorescens* and *S. liquefaciens*. TGE agar (TGE broth plus 1.5% agar) was used to culture *L. mesenteroides* and *L. sake* for enumeration. The plates were incubated at 30°C for 2 days for *P. fluorescens* and *S. liquefaciens* and for 3 days for *L. sake* and *L. mesenteroides*. For the pathogens the plates were incubated at 37°C for 2 days and then enumerated. The average of four counts (duplicate samples and duplicate plates) was used.

RESULTS

Viability loss of the pathogens by the combined effect of pressure, time, and temperature. The cell suspensions of the four pathogens were exposed to different combinations of pressure between 138 and 345 MPa for 5 to 15 min at 25 to 50°C. The control (nonpressurized) and treated cell suspensions were enumerated to determine viability loss under different treatment conditions. The population of all four pathogens decreased as the combinations of pressure,

time, and temperature were increased (Table 1). At 138 MPa the reduction in population or viability loss of all species of cells, except *L. monocytogenes*, was ≤ 1 log cycle at both 25° and 35°C even after 15 min of pressurization; at 45°C after 15 min *S. aureus* and *L. monocytogenes* showed 3.5- and 2.0-log-cycle reductions, respectively, while populations of the other two strains were reduced by about 1 log cycle. At 50°C, the viability loss of the pathogens at 138 MPa after 15 min ranged between 0.8 to 5.2 log cycles, with *E. coli* O157:H7 showing the least reduction. The viability loss of the pathogens at 207 MPa and 25 and 35°C remained below 2 log cycles after 15 min (except for *S. aureus*) and increased to about 4 to 6 log cycles after 15 min as the temperature was raised to 45°C (except for *S. typhimurium*). But at 50°C the population of all the pathogens was reduced by about 7 log cycles within 15 min of pressurization. The viability loss of the four pathogens increased as the pressure was increased to 276 MPa and at 50°C the populations of all the strains decreased by over 7.5 log cycles within 5 min. At 345 MPa, all four species showed 7.5- to 9.0-log-cycle reduction within 5 min both at 45 and 50°C and the viability loss of three of the species (not *L. monocytogenes*), reached over 7.5 log cycles even at 25°C in 15 min. *E. coli* O157:H7

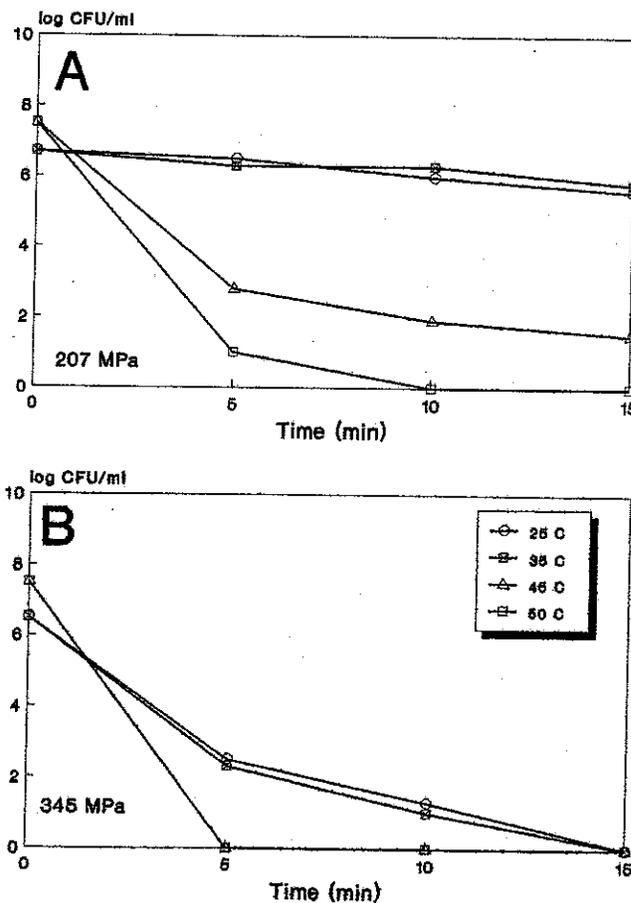


FIGURE 1. Rate of viability loss of *Escherichia coli* O157:H7 #932 at (A) 207 and (B) 345 MPa and 25 to 50°C during pressurization for 5 to 15 min. Initial rapid viability loss followed by tailing was observed either at 207 MPa and 45 and 50°C or at 345 MPa and 25 and 35°C. In general, a similar trend was found with the other three pathogens.

seemed to be relatively more resistant among the pathogens to pressurization at 345 MPa and 25 and 35°C. After 5 min of pressurization the death rate (except where viable cells could not be detected in 0.4 ml of cell suspensions) did not follow first-order kinetics. It was exponential initially, followed by a tailing effect, indicating that the rate of viability loss was at a maximum during the initial period of pressurization. This was especially evident when the cells were pressurized at and above 276 MPa at 25 and 35°C or at or below 207 MPa at 45 and 50°C (Fig. 1).

Viability loss of the spoilage bacteria by the combined effect of pressure, time, and temperature. The rate of viability loss of the four spoilage bacterial species under various conditions of pressurization is presented in Table 2. As before, the viability loss increased as the pressure, time and temperature were increased. At 138 MPa viability of both *L. sake* and *S. liquefaciens* was reduced by about 2 log cycles after pressurization for 15 min at 50°C. In contrast, *L. mesenteroides* and *P. fluorescens* were more sensitive to 138 MPa at 45 and 50°C; at 50°C the populations of *L. mesenteroides* and *P. fluorescens* were reduced by 6.0 and 7.3 log cycles, respectively, in 5 min. At 207 MPa and 45 to 50°C, three species (not *L. sake*) developed greater sensitivity. As the pressure increased to 276 MPa the viability losses varied greatly as can be observed from the following results: *L. sake*, 2.7 log cycles at 50°C in 15 min; *L. mesenteroides*,

6.9 log cycles at 50°C after 15 min; *S. liquefaciens*, 8 log cycles in 5 min at 50°C and in 10 min at 45°C; and *P. fluorescens*, 7.1 log cycles in 5 min at 25 to 50°C. At 345 MPa the populations of *S. liquefaciens* and *P. fluorescens* were reduced by over 7 log cycles within 5 min at 25 to 50°C. More than an 8-log-cycle reduction at 345 MPa was achieved for *L. mesenteroides* at 45 and 50°C in 10 min and for *L. sake* at 50°C in 15 min. As with the pathogens, the death rate kinetics showed an initial exponential rate followed by tailing, especially at lower pressure and/or lower temperature ranges (Fig. 2).

Decimal reduction (D) values. Although the rates of viability loss of the bacterial species studied were not linear with time and under several conditions of pressurization the viable cells could not be detected after 5 min, an attempt was made to calculate the D values for the eight species. Absolute values of the inverse slope of linear regressions between log₁₀ CFU and time were used for D value calculation (2). Where no survivors were detected after 5 min of pressurization, D values were estimated by dividing the initial log₁₀ CFU by 5. The D values at 345 MPa between 25 to 50°C are presented in Table 3. Among the eight species, D values at 25 to 50°C ranged between 0.6 and 10.1 min. At 45 and 50°C, the D values ranged between 0.6 and 3.5 and 0.6 and 1.9 min, respectively. Among the four pathogens D values ranged between 0.6 and 0.7 min at both

TABLE 2. Survivors of four foodborne spoilage bacteria following combined treatment of hydrostatic pressure, time, and temperature

Bacterial species	Temperature (°C)	Mean log ₁₀ CFU/ml (n = 2) following pressurization at											
		138 MPa for			207 MPa for			276 MPa for			345 MPa for		
		5	10 min	15	5	10 min	15	5	10 min	15	5	10 min	15
<i>Lactobacillus sake</i> FM1	25	7.3 (7.8) ^a	7.2	7.1	7.5 (8.0)	7.4	7.4	7.3 (7.7)	7.2	7.1	7.1 (8.0)	6.9	6.8
	35	7.2	7.1	7.0	7.3	7.3	7.2	7.1	7.0	7.0	7.0	5.8	4.1
	45	7.1	7.1	7.1	7.0	6.7	6.7	6.8	6.7	6.2	5.3	2.8	2.6
	50 ^c	6.3	6.2	5.8	6.2	6.2	6.1	5.9	5.8	5.0	5.2	2.2	ND ^b
<i>Leuconostoc mesenteroides</i> Ly	25	8.4 (9.0)	8.1	8.0	7.8 (8.8)	7.7	6.6	7.0 (8.6)	6.5	6.2	6.0 (8.7)	5.0	4.0
	35	8.1	8.0	7.9	7.1	6.5	5.6	6.2	6.1	6.1	5.3	3.9	3.5
	45	6.1	6.1	5.7	4.3	4.1	3.9	3.9	3.2	3.0	3.0	ND	ND
	50	3.0	3.0	2.7	3.1	2.8	2.0	3.0	2.8	1.7	1.6	ND	ND
<i>Serratia liquefaciens</i> FM 1	25	7.9 (8.1)	7.8	7.8	7.7 (8.0)	7.1	7.0	4.1 (8.0)	4.1	2.6	ND (7.9)	ND	ND
	35	7.7	7.6	7.1	7.2	7.1	7.0	2.8	1.9	1.0	ND	ND	ND
	45	7.0	6.7	6.6	4.2	4.0	2.8	1.0	ND	ND	ND	ND	ND
	50	7.0	6.1	5.9	1.0	ND	ND	ND	ND	ND	ND	ND	ND
<i>Pseudomonas fluorescens</i> FM 1	25	5.8 (7.3)	5.3	5.2	4.9 (7.8)	4.0	3.8	ND (7.1)	ND	ND	ND (7.8)	ND	ND
	35	5.3	5.1	5.1	4.8	4.0	3.6	ND	ND	ND	ND	ND	ND
	45	4.5	4.2	3.7	2.7	ND	ND	ND	ND	ND	ND	ND	ND
	50	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

^a Values in parentheses represent unpressurized control samples for a data set of time and temperature at one pressure.

^b ND, no CFU detected in total of 0.4 ml from duplicate samples. Each value is mean of duplicate samples.

^c Heating at 50°C for 15 min in 0.1% peptone solution reduced viability of the four spoilage bacteria by <1 to 4.2 log cycles with *L. mesenteroides* showing the least reduction (0.9 log cycle). Viability loss of bacterial cells by the combined treatment of pressure and temperature is not additive of individual treatments.

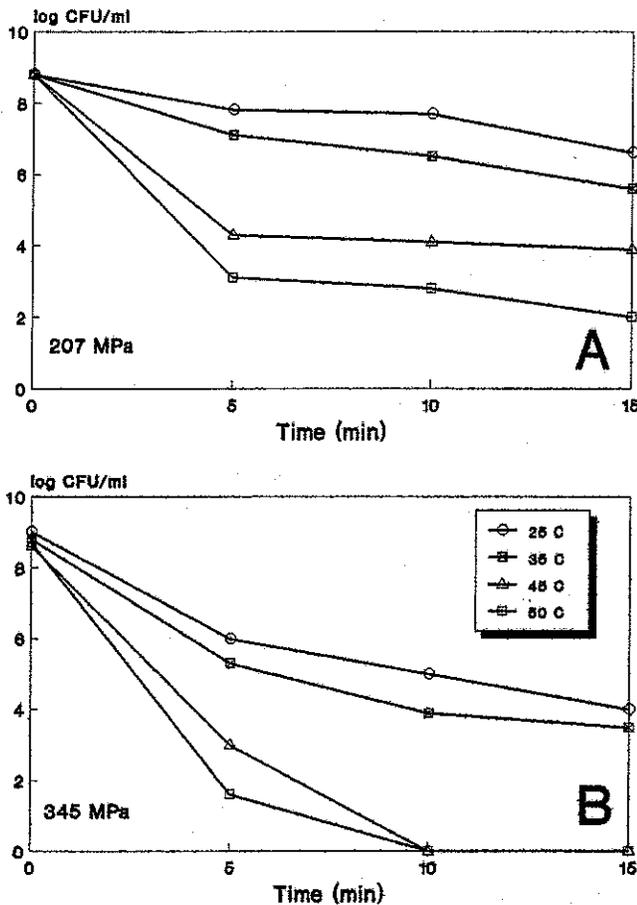


FIGURE 2. Rate of viability of *Leuconostoc mesenteroides* Ly at (A) 207 MPa and (B) 345 MPa at 25 to 50°C during pressure for 5 to 15 min. For other explanation see description in Figure 1.

45° and 50°C, with *E. coli* O157:H7 having the highest value. *D* values varied greatly for the four spoilage bacteria. While *S. liquefaciens* and *P. fluorescens* had the lowest *D* values of 0.6 min at 25°C, *L. mesenteroides* and *L. sake* had *D* values as high as 1.1 and 1.9 min, respectively, at 50°C.

Viability loss by pressurization in the presence of pediocin Ach. In an initial study, cell suspensions of the eight strains ($\geq 10^8$ CFU/ml) were mixed with pediocin Ach (final concentration 3,000 AU/ml) and pressurized at 345 MPa and 50°C for 5 min with no survivors detected (data not presented). In an effort to estimate the beneficial effect of adding pediocin Ach prior to pressurization, the cell suspensions with pediocin Ach were pressurized to 345 MPa for 5 min at 25°C and enumerated for viable cells. The results presented in Table 4 show viability loss increased when pediocin Ach was included during pressurization. The increases (in log cycles) were 1.7 for *S. aureus*, 2.1 for *L. monocytogenes*, 0.6 for *E. coli* O157:H7, 1.5 for *L. sake*, and 0.3 for *L. mesenteroides*.

DISCUSSION

Several low pH–low protein fruit products preserved by ultrahigh hydrostatic pressure have been commercially marketed (8). There is an interest in producing hydrostatic pressure-preserved foods from animal and plant sources that are high in pH (≥ 5.0) and protein. To retain acceptance

quality and to ensure the safety and shelf life of these products and to make the process economically feasible it is necessary to reduce populations of important bacteria by a desirable level (about log 10^7 to 10^8) at a moderate hydrostatic pressure. To achieve such a high level of destruction of foodborne pathogens and spoilage bacteria we combined four treatment parameters, namely, moderate hydrostatic pressure, pressurization time and temperature, and a bacteriocin, pediocin Ach.

The death rate kinetics of the four pathogens in this study are similar to those reported for these pathogens by other researchers (14, 15, 18, 20). There was very little death at lower pressure ranges (207 MPa) and at lower temperatures ($\leq 35^\circ\text{C}$), even after pressurizing for 15 min. As the pressure was increased above 207 MPa or the temperature raised above 35°C the population decrease followed an exponential rate for the first 5 min followed by tailing. An increase in the death rate by pressurization above 35°C is

TABLE 3. Estimated decimal reduction time (*D*) of eight pathogenic and spoilage bacterial species at 345 MPa and 25 to 50°C

Bacterial species	Temperature (°C)	<i>D</i> (min)
<i>Staphylococcus aureus</i> 582	25	1.7 ^a
	35	0.6
	45	0.6
	50	0.6
<i>Listeria monocytogenes</i> Scott A	25	4.0 ^a
	35	1.1 ^a
	45	0.6
	50	0.6
<i>Salmonella typhimurium</i> ATCC 14028	25	0.6
	35	0.6
	45	0.6
	50	0.6
<i>Escherichia coli</i> O157:H7 #932	25	2.0 ^a
	35	2.0 ^a
	45	0.7
	50	0.7
<i>Lactobacillus sake</i> FM1	25	10.1 ^a
	35	4.5 ^a
	45	3.5 ^a
	50	1.9
<i>Leuconostoc mesenteroides</i> Ly	25	5.6 ^a
	35	4.7 ^a
	45	1.1
	50	1.1
<i>Serratia liquefaciens</i> FM1	25	0.6
	35	0.6
	45	0.6
	50	0.6
<i>Pseudomonas fluorescens</i> FM1	25	0.6
	35	0.6
	45	0.6
	50	0.6

^a *D* value was calculated from the absolute value of the inverse of the slope from linear regression between logarithm of survivors and times (2). In the absence of any survivors, *D* value was estimated by dividing the initial population (in log₁₀) by time (min). Each value is the mean of four counts.

TABLE 4. Survivors of the bacteria following pressurization at 345 MPa at 25°C for 5 min in the absence and presence of pediocin AcH

Bacterial strain	Treatment ^a	Log CFU/ml
<i>Staphylococcus aureus</i> 582	Cont	8.4
	HP	1.7
	HP + Ped ^c	ND ^b
<i>Listeria monocytogenes</i> Scott A	Cont	8.1
	HP	6.8
	HP + Ped	4.7
<i>Salmonella typhimurium</i> ATCC 14028	Cont	8.1
	HP	ND
	HP + Ped	ND
<i>Escherichia coli</i> O157:H7	Cont	8.2
	HP	4.4
	HP + Ped	3.8
<i>Lactobacillus sake</i> FM1	Cont	8.1
	HP	5.8
	HP + Ped	4.3
<i>Leuconostoc mesenteroides</i> FM1	Cont	8.3
	HP	0.3
	HP + Ped	ND
<i>Serratia liquefaciens</i> FM1	Cont	8.3
	HP	ND
	HP + Ped	ND
<i>Pseudomonas fluorescens</i> FM1	Cont	7.2
	HP	ND
	HP + Ped	ND

^a Cont: unpressurized control; HP: pressurized only; HP + Ped: pressurized in the presence of 3,000 AU/ml pediocin AcH. Each value is mean of four counts.

^b ND: no CFU detected in 0.4 ml of the cell suspensions.

^c Pediocin AcH (3,000 AU/ml) alone reduced viability of unpressurized gram-positive bacteria by 0.7 to 3.4 log cycles. Unstressed gram-negative bacteria are not sensitive to pediocin AcH. Viability loss by the combined treatment is not additive of individual treatments.

related to a phase transition of bacterial cell membrane lipids (12). At 345 MPa and 50°C the death rate followed first-order of kinetics for all four pathogens and produced over a 7.5- to 9.0-log population decrease within 5 min.

The rate of viability loss of the four spoilage bacteria was low at 207 MPa and ≤35°C up to 15 min but increased greatly with an increase in pressure and/or temperature. The death rate during the first 5 min was exponential. At 345 MPa and 50°C the viability loss of *P. fluorescens* and *S. liquefaciens* reached about 8 log cycles within 5 min. In contrast, similar treatment reduced the populations of *L. sake* by 2.8 log cycles and of *L. mesenteroides* by 7.1 log cycles in 5 min. An 8-log-cycle loss in population required 15 min for *L. sake* and 10 min for *L. mesenteroides*.

One of the objectives of this study was to determine if within 5 min a pressurization and temperature combination would cause about an 8-log-cycle viability loss of all eight species. From the *D* values, it became evident that even at 345 MPa and 50°C this could not be achieved with *E. coli* O157:H7, *L. sake*, and *L. mesenteroides* with respective *D*

values of 0.7, 1.9, and 1.1 min. However, when pediocin AcH was included at 3,000 AU/ml during pressurization an additional reduction of 3 to 4 log cycles was obtained at both 45 and 50°C at 345 MPa. By combining other antibacterial compounds, this reduction could be increased further (9) (unpublished data). We preferred to use 345 MPa at 50°C instead of 45°C along with pediocin AcH and other antibacterial compounds to ensure ≥8-log-cycle reduction of the important spoilage and pathogenic bacteria. Under commercial operations for pressurization of solid or liquid foods at 50°C, it will be necessary to prewarm a product to 50°C before it enters the pressure vessel. There are some food-borne spoilage and pathogenic bacteria that can multiply at 45°C, but at 50°C most cannot initiate growth during a short time of prewarming (<30 min). It is anticipated that the entire process will involve flash warming of the product (not exceeding 5 to 10 min for solid products and even less than 1 min for liquid products) followed by 5 min of pressurization and finally chilling rapidly (in about 5 min) prior to storage at low temperature (preferably between 1 and 4°C) for long shelf life and safety. Our recent studies have shown that under these conditions of pressurization a minimum of 8 log cycles of reduction was achieved when inoculating the spoilage and pathogenic bacteria in 10% peptone solution, beef broth, and several processed meat products.

ACKNOWLEDGMENT

This study was funded with a contract from the U.S. Army Natick Research, Development and Engineering Center, Sustainability Directorate (contract DAAK 60-93-K-0003).

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