POLISH JOURNAL OF FOOD AND NUTRITION SCIENCES
www.pan.olsztyn.pl/journal/
e-mail: joan@pan.olsztyn.pl
Pol. J. Food Nutr. Sci.
2008, Vol. 58, No. 4, pp. 419-424

EFFECT OF HIGH PRESSURE ON SELECTED BACTERIA AT SUBZERO TEMPERATURE

Edyta Malinowska-Pańczyk, Ilona Kołodziejska, Edward Dunajski

Department of Food Chemistry, Technology and Biotechnology, Chemical Faculty, Gdańsk University of Technology, Gdańsk

Key words: high pressure and subzero temperature, Gram-negative bacteria, Gram-positive bacteria, inactivation of microorganisms

The objective of these investigations was to determine the viability of selected Gram-negative and Gram-positive bacteria exposed to high pressure and subzero temperature, in the range of 60÷193 MPa and –5 ÷ –20°C, without freezing of water. The results showed that similarly to the process conducted at the temperature above 0°C, Gram-negative bacteria and cells in the exponential growth phase are more sensitive to pressure treatment than the Gram-positive bacteria and cells being in the stationary phase of growth. Variations in resistance of microorganisms to high pressure were observed not only among the different species of bacteria but also among the strains belonging to the same species. Both pressure-sensitive and pressure-resistant strains appeared within mesophilic, psychrotrophic and thermophilic bacteria. However, the tendency to greater pressure sensitivity of Gram-negative psychrotrophic and psychrophilic strains than of the Gram-negative mesophiles was emphasized. The temperature of growth influenced the microorganisms' sensitivity to pressure.

INTRODUCTION

High hydrostatic pressure is one of more effective methods of extending the shelf life of food, especially the one which is sensitive to changes in sensory properties and nutritive value when conventional thermal process is used.

The factors affecting the sensitivity of microorganisms to high pressure at plus temperatures are well known. The degree of pressure inactivation of microorganisms depends on the type of microorganism, pH and composition of media, as well as on the parameters of the process [Alpas *et al*., 2000; Jordan *et al.,* 2001; Kalchayanand *et al*., 1998a,b; Patterson *et al*., 1995]. The viability of microorganisms also depends on the temperature at which cells are treated with high pressure. They are usually the most resistant to high pressure at plus temperatures in the range of 20÷35°C [Alpas *et al*., 2000; Gervilla *et al*., 1997]. Limited available data also showed that higher level of inactivation of microorganisms exists in high pressure-subzero temperature conditions than in a certain range of temperatures above 0°C [Hashizume *et al*., 1995; Hayakawa *et al*., 1998; Kalichevsky *et al*., 1995; Luscher *et al*., 2004; Ludwig *et al*., 1992; Noma & Hayakawa, 2003; Ponce *et al*., 1998; Reyns *et al*., 2000; Shen *et al*., 2005]. However, the reported results were obtained most often only with singles strains and different parameters of pressurization were used, which made it difficult to compare these data.

Therefore, the objective of these investigations was to determine the effect of high pressure and subzero temperature in the range of 60÷193 MPa and –5 ÷ –20°C on the viability of a large set of Gram-negative and Gram-positive bacteria, moreover, being in different growth phases and differing in optimal temperatures of growth. The sensitivity of strains within the same species was studied as well. The effect of growth temperature on the high pressure inactivation of cells was determined in the case of psychrotrophic bacteria.

MATERIALS AND METHODS

Cultures and growth conditions

The bacterial strains used in this study are listed in Table 1.

Inocula of strains were prepared by inoculating 100 mL of the appropriate medium (Table 2) with 100 μL of liquid culture (at stationary phase of growth) and incubating with shaking at optimal conditions of growth (Table 2). Under these conditions the cells were in the stationary phase.

In order to obtain an appropriate growth phase of cells, 100 μL of the stationary-phase culture was inoculated into 100 mL of fresh medium and incubated with shaking at optimal temperature of growth for particular microorganisms (Table 2). The bacteria growth was measured by determination of optical density at 660 nm. Based on the experimental curves middle exponential and stationary phase were determined.

The same media with the addition of agar, and the same temperature of incubation were used for enumeration of viable CFU in pressure-treated samples and controls serially diluted in phosphate-buffered saline.

The media were purchased from BTL Sp. z o.o., Łódź, Poland.

Pressure treatment

The pressure was generated in the similar way as described Hayakawa *et al*. [1998] and based on the phenomenon that in a sealed vessel filled with water and kept at subzero temper-

Author's address for correspondence: Ilona Kołodziejska, Department of Food Chemistry, Technology and Biotechnology, Chemical Faculty, Gdansk University of Technology, G. Narutowicza 11/12, 80-952 Gdańsk, Poland; fax: (48 58) 347 26 94; e-mail: i.kolodziejska@chem.pg.gda.pl

TABLE 1. Microorganisms used in the experiments.

Gram-negative	*Escherichia coli* IBA 72 (ATCC 11105), *Escherichia coli* CCUG 11321, CCUG 41424, *Escherichia coli* Ec29/51, Ec160/59 Czech NCTC, *Escherichia coli* K-12 PCM 2560 (NCTC 10538), *Escherichia coli* isolates[1] from: pork meat (MW), milk (M), cod meat (7250), kefir (2140)
	Salmonella Typhimurium TA98[2]
	Proteus vulgaris Pv1[3], *Proteus mirabilis* Pm1[3]
	Serratia marcescens Sm1[3]
	Enterobacter aerogenes Ea1[3], *Enterobacter faecium* Ef1[3]
	Pseudomonas fluorescens WSRO 121, *Pseudomonas fragi* PCM 2124, *Pseudomonas putida* PCM 1856, *Pseudomonas fluorescens* L1[3]
	Thermus thermophilus HB-8, *Thermus rubber* DFM1279, *Thermus filiformis* DFM4687
Gram-positive	*Listeria innocua*[4] III_1, III_3
	Micrococcus lysodeikticus ATCC 4696
	Arthrobacter psychrolactophilus[5]
	Staphylococcus aureus PCM 2054 (ATCC 25923), PCM 2101 (ATCC 12598), *Staphylococcus aureus* DSM2569
	Deinococcus radiodurans DSM20539
	Enterococcus faecalis Enf[3], *Enterococcus hirae* Enh1[3]
	Sarcina S1[3]
	Bacillus subtilis Bs2[3], *Bacillus cereus* Bc1[3]

[1] kindly provided by the J.S.H. Laboratoria Sp. z o.o., Poland; [2] Department of Pharmaceutical Technology and Biochemistry, Gdansk University of Technology, Poland; [3] Department of Food Chemistry, Technology and Biotechnology, Gdansk University of Technology, Poland; [4] kindly provided by Department of Food Microbiology, University of Agriculture, Szczecin, Poland; [5] kindly provided by Marianna Turkiewicz from the Institute of Technical Biochemistry, Technical University of Lodz, Poland

atures, the increasing volume of the forming ice I generates internal pressure (Table 3). According to Bridgman [1912], high pressure reduces the freezing and melting points of water to a minimum of –22°C at 207.5 MPa. Therefore, above this temperature, the sample placed in a sealed vessel is affected by the pressure in unfrozen state.

TABLE 3. Relationship between temperature and pressure generated in the pressure vessel [Kalichevsky *et al*., 1995].

Temperature (°C)	Pressure (MPa)
–5	59.8
–10	110.9
–15	156.0
–20	193.3

The cells in a particular phase of growth were centrifuged at 1300×*g* for 20 min at 5°C, and the pellets were resuspended in phosphate-buffered saline (pH 7.0) to give viable counts of about $10^8 \div 10^9$ CFU/mL. Cell suspensions (3 mL of each) were placed in sterile glass test tubes. Each tube was sealed with a stopper without leaving any bubbles of air inside, and kept at 0°C before pressurization. Due the special design, the stopper holds the tightness of the tube and it is able to move, which allows to expose the sample to the generated pressure.

The equipment used to generate pressure during the experiments was designed and constructed in our Department. The glass tubes containing cell suspension and a metal spring were placed in a cylindrical, metal vessel filled with water. The vessel was closed without leaving any air bubbles inside. It was gradually immersed within 40 min with the closed side down, in a temperature-controlled bath containing a mixture of ethanol, propylene glycol and distilled water (1:1:1, v/v) as the coolant. After pressure treatment at –5, –10, –15, and –20°C, half of the vessel was raised and warmed in the upper part to 15°C measured with a thermocouple. Then the vessel was taken out and placed for a few minutes in a water bath at 20°C. The total time of decompression did not exceed 10 min.

The cell suspensions were stored in an ice bath before viable counts were determined. Unpressurized cell suspensions were enumerated as controls.

TABLE 2. Growth conditions of bacterial strains.

Strains	Medium	Temperature of growth (°C)	Time of growth (h)
E. coli, P. vulgaris, P. mirabilis, S. marcescens, S. Typhimurium TA98, *E. aerogenes, E. faecium, L. innocua, M. lysodeikticus, S. aureus, E. fecalis, E. hirae, Sarcina* S1, *B. subtilis, B. cereus*	TSBYE	37	24
P. fluorescens, P. fragi, P. putida	TSBYE	28	24
D. radiodurans	No. 1	28	48
Pseudoalteromonas 22B and 518, *Psychrobacter, A. psychrolactophilus*	No. 2	15 15	72 72
T. thermophilus	No. 3	70	48
T. filiformis	No. 4	70	48
T. ruber	No. 5	55	48

TSBYE – tryptone soy broth supplemented with 0.6 g/L yeast extract; Medium: **No. 1:** yeast extract (1 g), peptone (5 g), glucose (1 g), distilled water (1000 mL); **No. 2:** yeast extract (1 g), peptone (2 g), lactose (10 g), sea salt (21.2 g), distilled water (1000 mL); **No. 3:** yeast extract (4 g), peptone (8 g), NaCl (2 g), distilled water (1000 mL); **No. 4:** yeast extract (1 g), tryptone (1 g), nitriloacetic acid (100 mg), $CaSO_4x2\ H_2O$ (40 mg), $MgCl_2x6\ H_2O$ (200 mg), 0.01 mol/L ferric (II) citrate (0.5 mL), trace elements solution (0.5 mL), 0.05 mol/L phosphate buffer – pH 7.2 (100 mL), distilled water (1000 mL). Trace elements solution contained: H_2SO_4 (0.5 mL), $MnSO_4xH_2O$ (2.28 g), $ZnSO_4x7\ H_2O$ (0.5 g), H_3BO_3 (0.5 g), $CuSO_4x5\ H_2O$ (25 mg), $Na_2MoO_4x6\ H_2O$ (25 mg), $CoCl_2x6\ H_2O$ (45 mg), distilled water (1000 mL). **No. 5:** yeast extract (1 g), peptone (5 g), soluble starch (1 g), distilled water (1000 mL).

The data presented in the tables and figures are mean values obtained from three independent experiments. The bars on the figures indicate the mean standard deviations for the data points. Some results were evaluated with the Student's t-test.

RESULTS AND DISCUSSION

Factors affecting viability of some bacteria under high pressure at subzero temperature

Growth stage

Microorganisms in the exponential phase of growth are usually more sensitive to the destructive effect of different stress factors than in the stationary phase of growth. The bacterial cells in the stationary phase are able to develop defensive mechanisms and then they become more resistant to stress conditions. Such a relationship, shown by some authors studying the effect of high pressure at plus temperatures on different types of bacteria [Benito *et al*., 1999; O'Reilly *et al.,* 2000; Pagan & Mackey, 2000], also exists when bacterial cells are pressurized at subzero temperatures. Three tested species differing in optimal temperature of growth: *P. fluorescens* WSRO121, *E. coli* IBA72, and *T. thermophilus* HB-8, were more sensitive to pressure in the exponential phase of growth than those from the stationary phase (Figure 1). Differences in the viability between cells in both phases of growth were more distinct when pressure above 111 MPa was used. For example, living cells of *T. thermophilus* in the exponential phase of growth were not detected after pressure treatment at 193 MPa and –20°C, while the exponential phase cells were inactivated by 4.5 log cycles.

It was confirmed in the experiments made on the larger set of Gram-negative bacteria belonging to different species that the stationary phase cells are more pressure resistant than the cells of the exponential phase (Table 4).

Optimal growth temperature

There are relatively few literature data on the pressure sensitivity of microorganisms dependence on their optimal temperature of growth. According to results reported by Gervilla *et al*. [1997, 1999] psychrotrophic *P. fluorescens* was more sensitive to high pressure than mesophilic *E. coli*. However, our preliminary experiments showed that among three tested species of bacteria, representatives of psychrotrophic, mesophilic and thermophilic organisms, the most resistant to pressure in the exponential phase of growth was *P. fluorescens* WSRO121, while the most sensitive was *T. thermophilus* (Figure 1). The number of viable cells of *P. fluorescens* WSRO121 and *E. coli* IBA72 after pressure treatment at 193 MPa and –20°C decreased by about 4.5 and 6.5 log cycles while alive cells of *T. thermophilus* were not detected under these conditions. Differences in pressure sensitivity among the tested species were less evident in the case of the stationary phase cells. Results of the experiments in which more bacterial species belonging to the particular type of microorganisms were studied showed large variations in pressure sensitivity among the species. As is presented in Table 4, both pressure sensitive and relatively pressure resistant species appeared within mesophilic, psychrotrophic and thermophilic bacteria. However, the tendency to the greater pressure sensitivity of Gram-

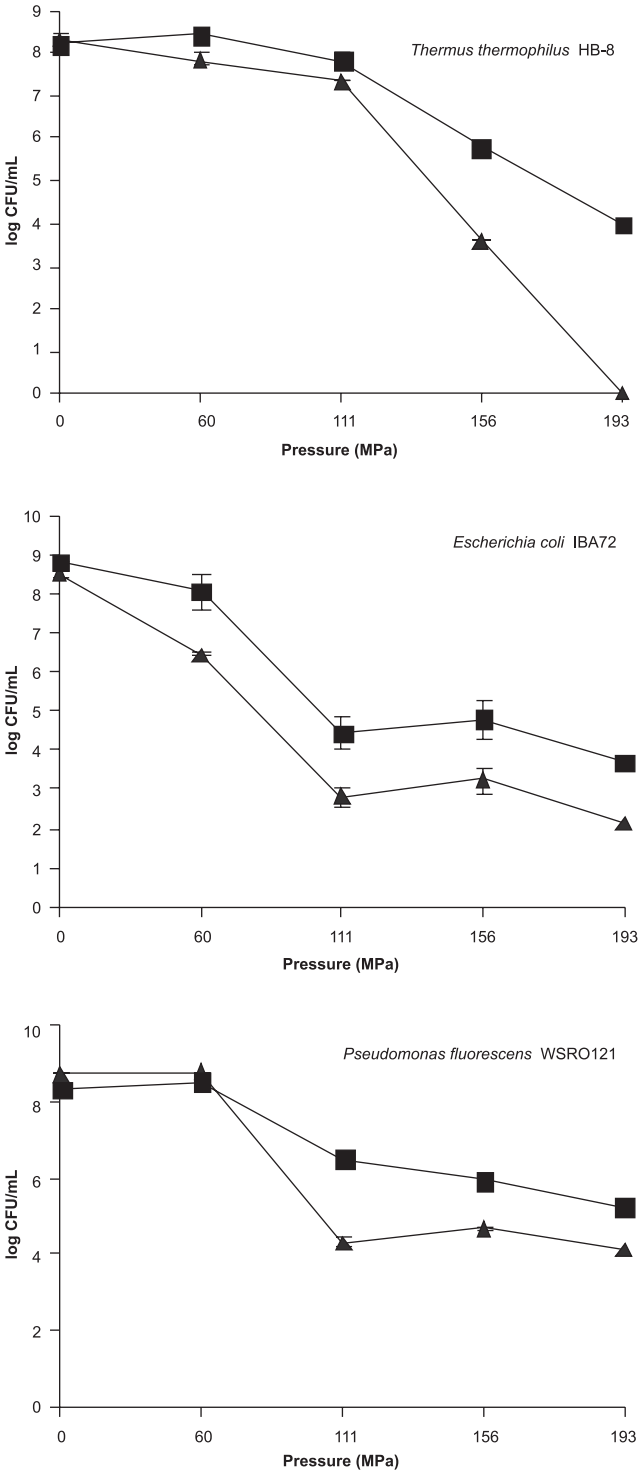

FIGURE 1. The effect of high pressure at subzero temperature on the viability of selected bacteria in the exponential phase (▲–▲) and stationary phase (■–■).

-negative psychrotrophic and psychrophilic (and probably thermophilic) species than of the Gram-negative mesophiles was emphasized. There were statistically significant differences in the viability of these bacteria (Table 5).

Gram-positive and Gram-negative bacteria

Numerous data showed that at positive temperatures Gram-positive bacteria in the stationary phase are more re-

TABLE 4. Viability loss of Gram-negative bacteria in the exponential and stationary phase after pressure treatment at 193 MPa and –20°C.

Bacterial strains	log N_0/N	
	Exponential phase	Stationary phase
Psychrophiles and psychrotrophs		
P. fluorescens WSRO121	4.5±0.8	3.6±0.8
P. fluorescens L-1	8.3±0.0	6.9±0.1
P. putida PCM 1856	7.0±0.1	5.5±0.1
P. fragi PCM 2124	7.1±0.1	5.2±0.1
Pseudoalteromonas 22B	8.3±0.0	6.3±0.8
Pseudoalteromonas 518	8.7±0.0	7.2±0.1
Psychrobacter 20	8.0±0.0	6.5±0.1
Mesophiles		
E. coli CCUG41424	1.2±0.1	0.9±0.1
E. coli IBA72	6.5±0.3	5.0±0.5
P. vulgaris Pv1	5.8±0.6	4.8±0.4
P. mirabilis Pm1	2.3±01	0.9±0.2
S. marcescens Sm1	4.9±0.3	4.8±0.1
S. Typhimurium TA98	5.0±0.2	1.8±0.1
E. aerogenes Ea1	2.0±0.1	1.0±0.1
E. feacium Ef1	5.0±0.3	3.2±0.1
Thermophiles		
T. thermophilus HB-8	8.1±0.0	4.4±0.4
T. rubber DFM1279	3.0±0.3	2.0±0.3
T. filiformis DFM4687	7.8±0.0	7.9±0.0

N – the number of cells detected after pressurization; N_0 – the number of cells in the control.

TABLE 5. Comparison of viability of psychrophiles or psychrotrophs and mesophiles[1] after pressure treatment at 193 MPa and –20°C.

Group of bacteria	Log N_0/N			
	Gram-negative		Gram-positive	
	Exponential phase	Stationary phase	Exponential phase	Stationary phase
Psychrophiles and psychrotrophs	7.4[a]	5.9[a]	5.0[a]	1.2[a]
Mesophiles	4.1[b]	2.8[b]	3.4[a]	1.6[a]

[1]The values for a particular column followed by different letters differ significantly ($p<0.05$).

sistant to pressure than the Gram-negative bacteria [Alpas *et al*., 2000; Arroyo *et al.,* 1997, 1999; O'Reilly *et al*., 2000]. Our results confirmed that the same relationship occurred when tested bacteria in the exponential and stationary phase were treated with high pressure at subzero temperatures (Table 6). There were statistically significant differences between Gram-negative (Table 4) and Gram-positive bacteria (Table 6) in spite of differences in resistance to pressure among species belonging to the same Gram-type.

Among the tested bacteria the most resistant to pressure were *S. aureus* strains. The cells were not inactivated under

TABLE 6. Viability loss of Gram-positive bacteria in the exponential and stationary phase after pressure treatment at 193 MPa and –20°C.

Bacterial strains	log N_0/N	
	Exponential phase	Stationary phase
Psychrophiles and psychrotrophs		
D. radiodurans	3.2±0.1	1.9±0.3
A. psychrolactophilus	5.2±0.2	0.2±0.1
L. innocua III_1	5.5±0.4	1.4±0.1
L. innocua III_3	6.3±0.1	1.4±0.1
Mesophiles		
S. aureus PCM 2054	0.8±0.1	0.6±0.1
S. aureus PCM 2101	1.3±0.1	0.1±0.1
S. aureus ATCC29213	0.1±0.1	0.3±0.1
M. lysodeikticus ATCC4696	4.7±0.1	0.9±0.3
E. faecalis Enf	5.6±0.3	2.9±0.1
E. hirae Enh1	1.6±0.2	0.1±0.1
Sarcina S1	1.3±0.4	0.2±0.1
B. subtilis Bs2	7.3±0.0	4.2±0.1
B. cereus Bc1	7.5±0.0	5.0±0.2

N – the number of cells detected after pressurization; N_0 – the number of cells in the control.

the pressure of 193 MPa at –20°C neither in the exponential phase nor in the stationary phase of growth. However, our results and the results of other authors [Patterson *et al.*, 1995] show that pressure-resistant strains may occur among Gram-negative bacteria. For example, *E. coli* CCUG41424 strain (Table 4) showed similar resistance to pressure as the cells of *S. aureus* (Table 6). On the other hand, within the *S. aureus* strains can exist ones that are very sensitive to pressure. Alpas *et al*. [1999] showed that among seven strains of *S. aureus* one was inactivated by about 7 log cycles after pressure treatment 345 MPa for 5 min at 25°C while other strains under these conditions survived completely.

According to Ludwig & Schreck [1997], the shape of cells determines their resistance to pressure independently of the Gram-type. The spherical forms of bacteria are more resistant than the rod-shaped ones. The results presented in Table 6 showed that at subzero temperature both tested *Bacillus* species in the exponential and in the stationary phase were more pressure-sensitive than spherical forms. The Gram-positive rods of *L. innocua* in the exponential phase were more sensitive to pressure than *S. aureus* strains. The number of viable cells of *L. innocua* decreased by 5–6 log cycles, while the cells of *S. aureus* were inactivated by less than 1 log cycle. However, the cells of both species in the stationary phase showed comparable resistance to pressure. On the other hand, spherical cells of *M. lysodeikticus,* similarly to *L. innocua*, were pressure-sensitive in the exponential phase of growth and pressure-resistant in the stationary phase. The stationary phase cells of *A. psychrolactophilus* were also resistant to pressure. In this phase they grow as Gram-positive cocci, while in the exponential phase elongated forms are formed. As shown in Table 6, in the exponential phase

of growth these cells were sensitive to pressure. However, the relationship between sensitivity to pressure and the shape and size of the bacterial cells must be confirmed on a larger number of particular groups of microorganisms.

Growth temperature

Some authors reported that cells in the stationary phase became more pressure-resistant and exponential cells more sensitive as the growth temperature increased [Casadei & Mackey, 1997; McClements *et al*., 2001]. Similar results were obtained in our experiments with *P. fluorescens* WSRO121 (Figure 2).

As shown in Figure 2, with the increase of the growth temperature intensification of differences in pressure sensitivity was observed between cells in the exponential and stationary phase of growth. When bacteria were grown at 26°C, the number of cells in the stationary and exponential phase decreased by about 2 and 4 log cycles, respectively, after treatment at 111 MPa and –10°C. There were no differences in the viability between cells of both growth phases when they grew at 4°C.

Variation in resistance to pressure among strains

Pressure sensitivity at subzero temperature was tested for ten strains of *E. coli*, including isolates from food. The differences in the resistance to high pressure among strains belonging to the same species occurred in cells of both the stationary and exponential phase (Table 7). The most sensitive in the exponential phase was *E. coli* MW isolated from pork. Even after pressure treatment at 60 MPa the number of viable cells decreased by about 5 log cycles and at 193 MPa living cells were not detected in the initial population of 10^8 cells/mL. The number of cells of all tested *E. coli* strains in the stationary phase was not reduced at pressure of 60 MPa. However, after pressure treatment at 193 MPa the differences in the viability of cells in the stationary phase occurred and the most sensitive was *E. coli* IBA72 strain. The number of viable cells of these bacteria decreased by *ca*. 5 log cycles (Table 7). Likewise, Pagan & Mackey [2000] showed differences in the sensitivity to pressure of three strains of *E. coli*. After pressure treatment for 8 min at 500 MPa and room temperature of the cells in the stationary phase, the number of *E. coli* NCTC 8003 and H1071 decreased by 6 and 4 log cycles, respectively. However, the third strain, *E. coli* C9490, was pressure-resistant under these conditions. When the cells of the three tested strains were exposed to pressure in the exponential phase of growth, there were no differences in resistance among them. In our experiments, differences in the sensitivity among strains of *E. coli* occurred in the exponential phase cells, but 5 of 10 tested strains showed similar viability under 193 MPa.

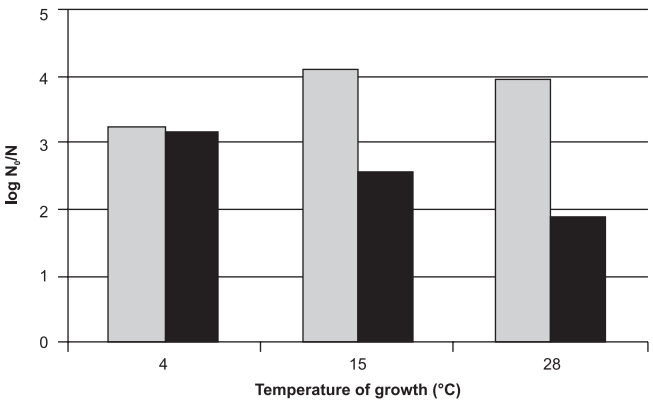

FIGURE 2. The effect of temperature growth on the inactivation of *P. fluorescens* WSRO 121 in the exponential phase ■ and stationary phase □ at 111 MPa and –10°C. (1) N – the number of cells detected after pressurization; N_0 – the number of cells in the control).

TABLE 7. Viability loss of *E. coli* strains in the exponential and stationary phase after pressure treatment at 60 MPa and –5°C or at 193 MPa and –20°C.

Bacterial strains	log N_0/N			
	Pressurized at		Pressurized at	
	60 MPa	193 MPa	60 MPa	193 MPa
	Exponential phase		Stationary phase	
E. coli 2140	2.0±0.2	3.2±0.2	0.5±0.1	0.8±0.4
E. coli 7250	1.9±0.2	7.1±0.6	0.2±0.1	0.3±0.0
E. coli MW	4.7±0.6	8.5±0.0	0.1±0.1	1.3±0.1
E. coli M	0.1±0.1	8.4±0.0	0.1±0.1	0.9±0.0
E. coli Ec160/59	0.1±0.0	5.8±0.3	0.0±0.1	0.5±0.1
E. coli K-12	2.4±0.2	5.4±0.1	0.1±0.1	0.4±0.5
E. coli IBA72	2.2±0.1	6.5±0.3	0.7±0.1	5.2±0.5
E. coli CCUG41424	1.0±0.1	1.2±0.1	0.1±0.1	0.7±0.1
E. coli CCUG11321	4.9±0.5	5.2±0.1	1.1±0.1	1.4±0.5
E. coli Ec27/52	5.1±0.1	5.1±0.1	0.6±0.3	0.7±0.1

N – the number of cells detected after pressurization; N_0 – the number of cells in the control.

Alpas *et al*. [1999] in the experiments with four species of bacteria, each including 6-9 strains of food-borne pathogens, showed distinc variation in pressure sensitivity among bacterial strains when pressurization was conducted at 20-25 °C. However the differences in the viability were greatly reduced at 50°C. It was found that a decrease of temperature below 20°C also increased the pressure sensitivity of microorganisms [Hashizume *et al.*, 1995; Ponce *et al*., 1998; Moussa *et al*., 2006]. Thus, it could be expected that differences in resistance to pressure among strains should be eliminated after pressure treatment at subzero temperature. However, our experiments did not confirm that, probably because the pressure of 193 MPa was too low.

CONCLUSIONS

In the process of pressure treatment of bacteria at subzero temperature without freezing of water the same relationships exist between viability of cells and their Gram type, shape, phase of growth as were shown by other authors in the process conducted above 0°C.

The maximum pressure obtainable in the sealed vessels at –20°C is not higher than *ca*. 200 MPa. Although this pressure is very effective in reducing the number of viable cells of some bacteria, it may be insufficient to complete inacti-

vation of the more pressure-resistant Gram-positive and Gram-negative bacteria. It seems that using reasonably high pressure in combination with antimicrobial factors is a promising method for achieving a desirable increase in the inactivation of bacteria.

ACKNOWLEDGEMENTS

This research project was financed by the national research budget in the years 2003–2006. The authors also wish to express their gratitude to Professor Zdzisław E. Sikorski for the final correction of this manuscript.

REFERENCES

1. Alpas H., Kalchayanand N., Bozoglu F., Ray B., Interactions of high hydrostatic pressure, pressurization temperature and pH on death and injury of pressure-resistant and pressure-sensitive strains of foodborne pathogens. J. Food Microbiol., 2000, 60, 33–42.
2. Alpas H., Kalchayanand N., Bozoglu F., Sikes A., Dunne P., Ray B., Variation in resistance to hydrostatic pressure among strains of food-borne pathogens. Appl. Environ. Microbiol., 1999, 65, 4248–4251.
3. Arroyo G., Sanz P.D., Prestamo G., Effect of high pressure on the reduction of microbial populations in vegetables. J. Appl. Microbiol., 1997, 82, 735–742.
4. Arroyo G., Sanz P.D., Préstamo G., Response to high-pressure, low temperature treatment in vegetables: determination of survival rates of microbial populations using flow cytometry and detection of peroxidase activity using confocal microscopy. J. Appl. Microbiol., 1999, 86, 544–556.
5. Benito A., Venbufftoura G., Casadei M., Robinson T., Mackey B., Variation in resistance of natural isolates of *Escherichia coli* O157 to high hydrostatic pressure, mild heat, and other stresses. Appl. Environ. Microbiol., 1999, 65, 1564–1569.
6. Bridgman P.W., Water in the liquid and five solid forms under pressure. Proc. Amer. Acad. Arts Sci., 1912, XLVII, 439–558.
7. Casadei M.A., Mackey B.M., The effect of growth temperature on pressure resistance of *Escherichia coli*. 1997, *in*: High Pressure Research in the Biosciences and Biotechnology (ed. K. Heremans). Leuven University Press, Leuven, pp. 281–282.
8. Gervilla R., Felipe X., Ferragut V., Guamis B., Effect of high hydrostatic pressure on *Escherichia coli* and *Pseudomonas fluorescens* strains in ovine milk. J. Dairy Sci., 1997, 80, 2297–2303.
9. Gervilla R., Mor-Mur M., Ferragut V., Guamis B., Kinetics of destruction of *Escherichia coli* and *Pseudomonas fluorescens* inoculated in ewe's milk by high hydrostatic pressure. Food Microbiol., 1999, 16, 173–184.
10. Hashizume C., Kimura K., Hayashi R., Kinetic analysis of yeast inactivation by high pressure treatment at low temperatures. Biosci. Biotechnol. Biochem., 1995, 59, 1455–1458.
11. Hayakawa K., Ueno Y., Kawamura S., Kato T., Hayashi R., Microorganism inactivation using high pressure generation in sealed vessels under sub-zero temperature. Appl. Microbiol. Biotechnol., 1998, 50, 415–418.
12. Jordan S.L., Pascual C., Bracey E., Mackey B.M., Inactivation and injury of pressure-resistant strains of *Escherichia coli* O157 and *Listeria monocytogenes* in fruit juices. J. Appl. Microbiol., 2001, 91, 463–469.
13. Kalchayanand N., Sikes A., Dunne C.P., Ray B., Factors influencing death and injury of foodborne pathogens by hydrostatic pressure-pasteurization. Food Microbiol., 1998a, 15, 207–214.
14. Kalchayanand N., Sikes T., Dunne C.P., Ray B., Interaction of hydrostatic pressure, time and temperature of pressurization and pediocin AcH on inactivation of foodborne bacteria. J. Food Prot., 1998b, 61, 425–431.
15. Kalichevsky M.T., Knorr D., Lillford P.J., Potential food applications of high-pressure effects on ice-water transitions. Trends Food Sci. Technol., 1995, 6, 253–259.
16. Luscher C., Balasa A., Frohling E., Ananta E., Knorr D., Effect of high-pressure-induced ice I-to-ice III phase transitions on inactivation of *Listeria innocua* in frozen suspension. Appl. Environ. Microbiol., 2004, 70, 4021–4029.
17. Ludwig H., Bieler K., Hallbauer K., Scigalla W., Inactivation of microorganisms by hydrostatic pressure. 1992, *in:* High Pressure and Biotechnology (eds. C. Balny, R. Hayashi, K. Heremans, P. Masson). John Libbey and Co., Ltd., London, pp. 25–32.
18. Ludwig H., Schreck Ch., The inactivation of vegetative bacteria by pressure. 1997, *in*: High Pressure Research in the Bioscience and Biotechnology (ed. K. Heremans). Leuven University Press, Leuven, pp. 221–224.
19. McClements J.M.J., Patterson M.F., Linton M., The effect of growth stage and growth temperature on high hydrostatic pressure inactivation of some psychrotrophic bacteria in milk. J. Food Prot., 2001, 64, 514–522.
20. Moussa M., Perrier-Cornet J.M., Gervais P., Synergistic and antagonistic effects of combined subzero temperature and high pressure on inactivation of *Escherichia coli*. Appl. Environ. Microbiol., 2006, 71, 150–156.
21. Noma S., Hayakawa I., Barotolerance of *Staphylococcus aureus* is increased by incubation at below 0 °C prior to hydrostatic pressure treatment. Int. J. Food Microbiol., 2003, 80, 261–264.
22. O'Reilly C.E., O'Connor P.M., Kelly A.L., Beresford T.P., Murphy P.M., Use of hydrostatic pressure for inactivation of microbial contaminants in cheese. Appl. Environ. Microbiol., 2000, 66, 4890–4896.
23. Pagan R., Mackey B., Relationship between membrane damage and cell death in pressure treated *Escherichia coli* cells: differences between exponential- and stationary- phase cells and variation among strains. Appl. Environ. Microbiol., 2000, 66, 2829–2834.
24. Patterson M.F., Quinn M., Simpson R., Gilmour A., Sensitivity of vegetative pathogens to high hydrostatic pressure treatment in phosphate-buffered saline and foods. J. Food Prot., 1995, 58, 524–529.
25. Ponce E., Pla R., Mor(Mur M., Gervilla R., Guamis B., Inactivation of *Listeria innocua* inoculated in liquid whole egg by high hydrostatic pressure. J. Food Prot., 1998, 61, 119–122.
26. Reyns K.M.F.A., Sootjens C.C.F., Cornelis K., Weemaes C.A., Hendrickx E., Michiels Ch.W., Kinetic analysis and modeling of combined high-pressure-temperature inactivation of the yeast *Zygosaccharomyces bailii*. Int. J. Food Microbiol., 2000, 56, 199–210.
27. Shen T., Urrutia Benet G., Brul S., Knorr D. Influence of high-pressure-low temperature treatment on the inactivation of *Bacillus subtilis* cells. Innov. Food Sci. Emerg. Technol., 2005, 6, 271–278.

Received December 2007. Revision received and accepted April 2008.