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Influence of kinetic parameters of high pressure processing on bacterial inactivation in a buffer system

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Abstract

High pressure processing is recently applied in the food industry to inactivate spoilage and pathogenic microorganisms. Bacterial cells exhibit various barosensibility, and the role of pressurization, depressurization and constant pressure stage remain unknown. We investigated the effect of high pressure processing on *Salmonella typhimurium* and *Listeria monocytogenes* cells at 400 and 500 MPa respectively in buffer pH 7 at 20 °C. We applied various pressurization/depressurization kinetic rates (1, 5 and 10 MPa/s for pressurization and 250, 20 and 5 MPa/s for depressurization), and various pulse series or pressure holding times.

Results show that high pressure pulses reduced linearly the number of bacterial cells according to the product of pressure and time: we defined this product as a Barometric Power (BP).

Reduction of both microorganisms increased when holding time increased from 5 to 20 min, and better results were obtained when the rate of pressurization and depressurization were increased.

Keywords: High pressure processing; Bacteria; *Listeria monocytogenes*; *Salmonella typhimurium*; Inactivation

1. Introduction

Nonthermal methods allow the processing of foods below the temperatures used during thermal pasteurization, so that flavours, essential nutrients and vitamins undergo minimal or no changes during processing. Foods can be nonthermally processed by different techniques such as irradiation, high hydrostatic pressure, ultrasounds, pulsed electric fields, light pulses, and oscillating magnetic fields. Due to technological developments, high pressure processing (HPP) has received increased attention during the last decade (Cheftel and Dumay, 1997; Torres and Velazquez, 2005).

Today high pressure processing constitutes a novel technology in the food industry for preserving food products against microbial and enzymatic degradation. Many authors after the works of Hite (1899) have studied the effect of high pressure on microorganism survival. Results show that the pressure sensitivity of bacteria depends on the pressure applied, and it is mainly the cell membrane which is affected by high pressure treatments (Smelt et al., 1994). Furthermore, pressurization induces changes in the cell morphology and biochemical reactions (Ritz et al., 2002, 2000). Microorganisms are more or less sensitive to pressure treatment especially depending on their biological structure (Hoover, 1993). The efficiency of a food preservation treatment is usually evaluated by its capacity to destroy pathogenic microorganisms in buffer systems (Shigehisa et al., 1991). Several authors have highlighted the efficiency of this novel process (Winckel et al., 1997; Murano et al., 1999).

Even though the treatment characteristics seem to play a major role in the inactivation of microorganisms, this aspect remains widely unknown. Hayakawa et al. (1998) showed that rapid decompression (1 ms) enhanced bacterial reduction of *Bacillus stearothermophilus* by high pressure treatment.

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The rapid decompression applied after continuous pressurization induced a fast adiabatic expansion of water and generated an impulsive force. According to Hayakawa et al. (1998), the combination of this impulsive force and pressurization causes much more bacterial inactivation than pressurization alone. Noma et al. (2002) investigated the effect of slow (30 s) and rapid depressurization (1 ms) on several vegetative bacteria: rapid depressurization led to a significantly more efficient inactivation of vegetative bacteria than slow depressurization. These results suggested that a rapid depressurization procedure could enhance the degree of pressure-mediated injury. Smelt (1998) assumed that slow pressurization might induce a stress response of the microbial cells and hence lead to a lower inactivation of bacteria. Rademacher et al. (2002) proved that inactivation of *Listeria innocua* by high pressure remained the same in case of fast pressurization (500 MPa/min) and slow depressurization (100 MPa/min) and vice versa. The influence of pressurization and depressurization kinetic parameters on inactivation rates has been much debated in the literature (Noma et al., 2002). The cost of high pressure equipment increases strongly when required high pressure level increases, so the determination of optimal high pressure conditions is of great importance (Hoover, 1993; Torres and Velazquez, 2005).

In the present study we evaluated the effect of pressurization and depressurization kinetic parameters during high pressure processing, on inactivation of two vegetative bacteria, *Listeria monocytogenes* and *Salmonella typhimurium*. At first, we studied the effect of pressurization and depressurization kinetic parameters, by the way of pulses, on cell viability. Then, we analyzed the impact of the treatment by testing successive pulses of pressure and several holding times.

2. Materials and methods

2.1. Bacterial strains

L. monocytogenes scottA and *S. typhimurium* were obtained from the Pasteur Institute collection (CIP, Paris, France) and stored at −30 °C in cryobeads (AES, Combourg, France).

2.2. Culture conditions and preparation of inoculum

The first bacterial culture was obtained by inoculating a cryobead into brain–heart infusion (BHI; Biokar, Beauvais, France) incubated at 37 °C for 24 h. This first culture was used to prepare a subculture by inoculating 1 per 1000 (v/v) into fresh BHI for a 18 h incubation at 37 °C. The pressurized samples were composed of 1 ml of subculture diluted in 9 ml of buffer and gave approximately 10^8 CFU/ml. The buffer used was a phosphate buffer (pH 7.0) composed of Na_2HPO_4 (0.2 mol l^{-1}) and NaH_2PO_4 (0.2 mol l^{-1}) (Merck, Darmstadt, Germany). The samples were placed in a sterile polyethylene bag (AES, Combourg, France).

2.3. High pressure processing: kinetic parameters of pressurization

High pressure processing was carried out in a 3.5 l reactor (ACB Pressure Systems, Nantes, France) equipped with temperature and pressure regulators (Fig. 1). The pressure level was set according to the bacterial strain: *S. typhimurium* was exposed to 400 MPa and *L. monocytogenes* to 500 MPa. Kinetic parameters were tested: 1, 5 and 10 MPa/s for the pressurization step and 250, 20 and 5 MPa/s for depressurization (Table 1).

Aluminium cylinders were used to reduce the volume of the pressure vessel (i.e. reduce time of pressurization) and avoid heating of the samples during pressurization (Fig. 1). All kinetic parameters of processing were combined in order to determine the better kinetic parameters for a maximum reduction of bacteria obtained (Tables 2 and 3). Then the effect of pulses (1, 2 and 3 pulses) and holding times (5, 10, 15 and 20 min) were studied with these parameters.

We considered the area under the curve of pressure versus time (Fig. 2). This area is defined by ∫Pdt, and can be calculated by the product of pressure and time for each part of the diagram. As the result is expressed in MPa.s, we can class this area as a Barometric Power per m^3, designated as BP. Temperature of the cooling jacket surrounding the pressure vessel was set at 20 °C during pressure treatment. One thermocouple K-type (0.3 mm diameter, Omega, Stamford, USA) positioned close to the sample allowed to follow the temperature variations during treatment. Temperature variation due to adiabatic heating effect during pressure increase was recorded by a data-logger (SA32 AOIP, Evry, France) every 1 or 5 s (Fig. 2). After pressure treatment, samples were stored on ice (maximum 4 h) until microbial analysis.

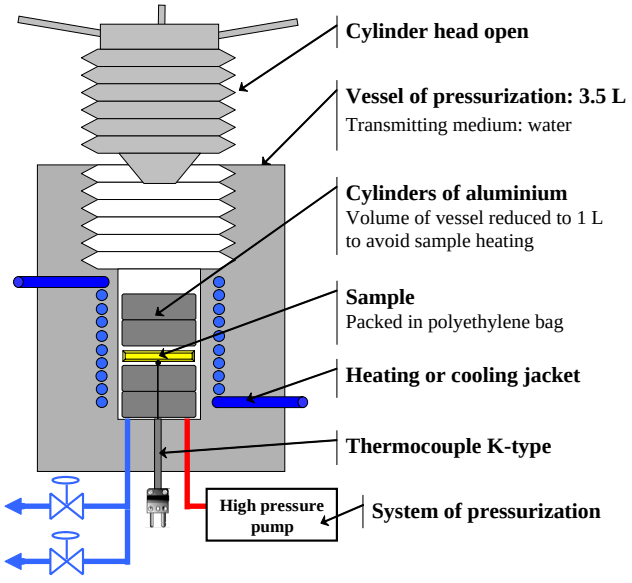

Fig. 1. Schema of the high pressure vessel from ACB pressure systems.

Table 1
Temperature profiles during high pressure processing according to kinetic parameters applied for treatment at 400 MPa (*Salmonella typhimurium*) and 500 MPa (*Listeria monocytogenes*)

Kinetic parameters		*Salmonella typhimurium* [1]Temperature (°C)		*Listeria monocytogenes* [1]Temperature (°C)	
Pressurization (MPa/s)	Depressurization (MPa/s)	At pressure	At the end	At pressure	At the end
1	250	23.5±0.1	14.5±0.2	25.6±0.2	12.5±0.2
1	20	24.1±0.3	15.2±0.3	25.9±0.1	13.2±0.1
1	5	24.0±0.2	15.3±0.2	25.8±0.1	13.7±0.1
5	250	27.6±0.2	18.5±0.2	27.4±0.2	17.7±0.1
5	20	27.2±0.1	18.8±0.1	28.0±0.1	18.2±0.3
5	5	28.2±0.1	18.7±0.3	28.9±0.3	17.2±0.2
10	250	29.0±0.2	18.5±0.3	29.7±0.2	18.2±0.3
10	20	29.4±0.3	18.2±0.1	29.2±0.2	17.5±0.3
10	5	29.1±0.2	18.9±0.2	29.8±0.3	17.0±0.2

[1]All values were means±standard deviation of three values.

2.4. Enumeration of viable L. monocytogenes and S. typhimurium

The number of cells was determined before and after treatment, and the reduction was calculated by the difference between these two counts in $\log_{10}$. Cells were enumerated by plating 0.1 ml volumes twice on plate count agar (PCA) (Biokar, Beauvais, France), which were then incubated for 48 h at 37 °C. For low numbers of viable cells, the entire sample (10 ml) was divided in two plates and poured with PCA. Thus, the threshold limit was 0.1 cfu/ml, corresponding to the result 0 mentioned on the figures.

2.5. Statistical analysis

All experiments were made in triplicate. The data of microbial reduction were the means of 3 replicates. Statistical analysis was performed using a one-way analysis of variance according to the general linear model procedure with least-square means effects to determine significant differences between treatments. Multiple range test was applied to determine which means were significantly different according to Fisher's Least Significant Differences (LSD). Significant differences of inactivation were determined with 5% level of significance ($P<0.05$) by Student's test. Statistical analysis was carried out using Statgraphics plus version 2.1 software (Statistical Graphics Corp., Princeton NJ, USA).

3. Results and discussion

3.1. Thermal conditions during treatment

Application of pressure induces an increase of temperature due to the adiabatic compression (Pehl et al., 2000). Table 1 shows the evolution of temperature profiles close to the sample during pressurization and depressurization. As a result we confirmed the observation made by Pehl et al. (2000) who showed that during compressing and decompressing, the change in the internal energy of the substance leads to a temperature variation. The extent of the temperature variation depends on the pressurization/depressurization kinetic parameters, kind of substance and geometry of the vessel. Thus, the

Table 2
Effects of high pressure processing on inactivation of *Salmonella typhimurium* in phosphate buffer pH 7 after pressurization at 400 MPa with various kinetic parameters of pressurization/depressurization

Treatment of *Salmonella typhimurium* at 400 MPa						
Pressurization		Depressurization				
Rate (MPa.s)	Time (s)	Rate (MPa.s)	Time (s)	Reduction $\log_{10}(N/N_0)$[a]	Barometric power (MPa.s)[b]	Efficiency of treatment[a,c]
1	400	250	–	$-1.13\pm0.23^{A/a}$	80000	$-1.41\cdot10^{-5\ A/a}$
1	400	20	20	$-1.09\pm0.12^{A/b}$	84000	$-1.29\cdot10^{-5\ A/a}$
1	400	5	80	$-1.19\pm0.23^{A/ab}$	96000	$-1.24\cdot10^{-5\ A/a}$
5	80	250	–	$-0.36\pm0.33^{B/a}$	16000	$-2.22\cdot10^{-5\ A/a}$
5	80	20	20	$-0.56\pm0.13^{B/b}$	20000	$-2.79\cdot10^{-5\ A/a}$
5	80	5	80	$-0.63\pm0.18^{B/ab}$	32000	$-1.97\cdot10^{-5\ A/a}$
10	40	250	–	$-0.12\pm0.12^{B/a}$	8000	$-1.52\cdot10^{-5\ A/a}$
10	40	20	20	$-0.19\pm0.12^{B/b}$	12000	$-1.62\cdot10^{-5\ A/a}$
10	40	5	80	$-0.59\pm0.39^{B/ab}$	24000	$-2.46\cdot10^{-5\ A/a}$

The survival of microorganisms was enumerated on PCA.
Capital letters (A, B) in the same column indicate no significant differences ($P<0.05$) of pressurization parameters and small letters (a, b) in the same column indicate no significant differences ($P<0.05$) of depressurization.
[a] All values were means±standard deviation of three values.
[b] Barometric power is area defined by $\int Pdt$, and can be calculated by the product of pressure and time for each part of the diagram.
[c] Efficiency of treatment was the ratio of reduction $\log_{10}(N/N_0)$ to barometric power (MPa.s).

Table 3
Effects of high pressure processing on inactivation of *Listeria monocytogenes* in phosphate buffer pH 7 after pressurization at 500 MPa with various kinetic parameters of pressurization/depressurization

Treatment of *Listeria monocytogenes* at 500 MPa						
Pressurization		Depressurization				
Rate (MPa.s)	Time (s)	Rate (MPa.s)	Time (s)	Reduction $\log_{10}(N/N_0)$[a]	Barometric power (MPa.s)[b]	Efficiency of treatment[a,c]
1	500	Quick	–	$-0.37\pm0.09^{A/a}$	125 000	$-2.92\cdot10^{-6}$ A/a
1	500	20	25	$-0.32\pm0.06^{A/a}$	131 250	$-2.23\cdot10^{-6}$ A/a
1	500	5	100	$-0.54\pm0.03^{A/a}$	150 000	$-3.62\cdot10^{-6}$ A/a
5	100	Quick	–	$-0.06\pm0.24^{B/a}$	25 000	$-2.32\cdot10^{-6}$ A/a
5	100	20	25	$-0.12\pm0.05^{B/a}$	31 250	$-3.91\cdot10^{-6}$ A/a
5	100	5	100	$-0.19\pm0.01^{B/a}$	50 000	$-3.83\cdot10^{-6}$ A/a
10	50	Quick	–	$0.05\pm0.21^{C/a}$	12 500	$4.30\cdot10^{-6}$ A/a
10	50	20	25	$0.00\pm0.17^{C/a}$	18 750	$0.56\cdot10^{-7}$ A/a
10	50	5	100	$-0.00\pm0.08^{C/a}$	37 500	$-0.12\cdot10^{-6}$ A/a

The survival of microorganisms was enumerated on PCA.
Capital letters (A, B) in the same column indicate no significant differences ($P<0.05$) of pressurization parameters and small letters (a, b) in the same column indicate no significant differences ($P<0.05$) of depressurization.
[a] All values were means ± standard deviation of three values.
[b] Barometric power is area defined by ∫Pdt, and can be calculated by the product of pressure and time for each part of the diagram.
[c] Efficiency of treatment was the ratio of reduction $\log_{10}(N/N_0)$ to barometric power (MPa.s).

most important variations were observed for 10 MPa/s pressurization and 250 MPa/s depressurization. Fig. 2 shows an increase of +9 °C during pressurization treatment at 400 MPa for *S. typhimurium* and +10 °C for *L. monocytogenes* at 500 MPa. Concerning depressurization the most significant reduction of temperature (12.5 °C) was obtained in the case of heating associated with a low pressurization at the slowest rate (1 MPa/s). Fig. 2 presents the temperature profiles during processing with pulse series and holding times for the highest pressure treatment (500 MPa, *L. monocytogenes*) with various kinetics parameters. For holding time, the sample temperature decreased during pressure application and after 15 min the temperature fell to 20 °C, due to the effect of the cooling jacket. When pressure was released slowly the product

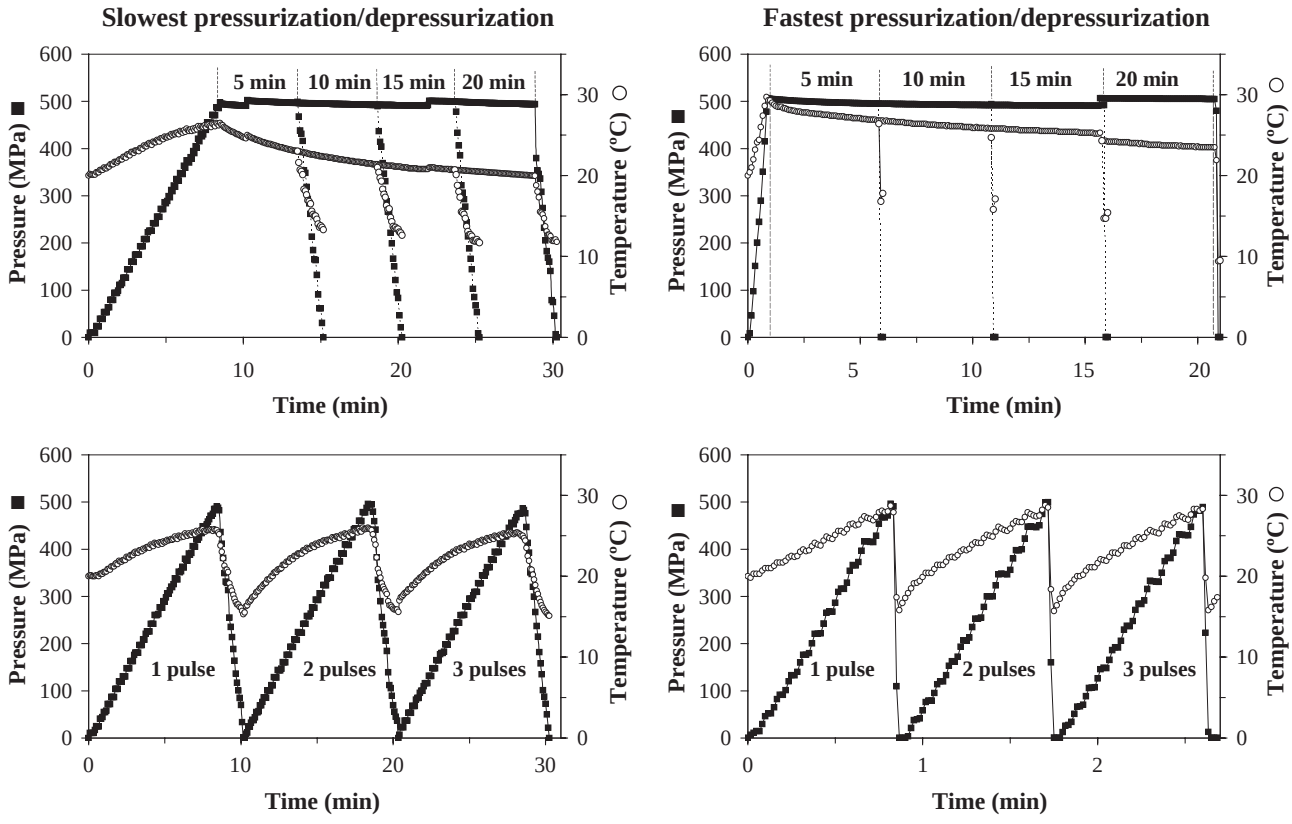

Fig. 2. Temperature and pressure for 500 MPa processing, with the slowest and fastest parameters of pressurization/depressurization on pulse series (1, 2 and 3) and holding time (5, 10, 15, 20 min), ■ pressure in the vessel, ○ temperature near the sample. Each figure is composed by the combination of all the possible treatment applied in the same pressure conditions.

Table 4
Effects of high pressure processing conditions, pulse series (1, 2 or 3) and various holding times (5, 10, 15 and 20 min) on inactivation of *Salmonella typhimurium* in phosphate buffer pH 7 after pressurization at 400 MPa with different kinetic parameters of pressurization/depressurization

Treatment of *Salmonella typhimurium* at 400 MPa				
		Time of treatment (s)	Barometric power (MPa.s)	Reduction $\log_{10}(N/N_0)$[a]
Slowest kinetic parameters: (pressurization at 1 MPa/depressurization at 5 MPa/s)				
Number of pulses	1	480	96 000	−1.31±0.07[a]
	2	960	192 000	−3.90±0.64[b]
	3	1440	288 000	−5.79±0.03[c]
Holding time at 400 MPa	5 min	780	212 000	−5.29±0.67[c]
	10 min	1080	332 000	−7.24±0.58[d]
	15 min	1380	452 000	−7.59±0.34[d]
	20 min	1680	572 000	−7.59±0.34[d]
Fastest kinetic parameters: (pressurization at 10 MPa/quick depressurization < 2 s)				
Number of pulses	1	40	8000	0.26±0.15[a]
	2	80	16 000	0.09±0.01[a]
	3	120	24 000	−1.96±0.00[b]
Holding time at 400 MPa	5 min	340	128 000	−7.00±0.61[c]
	10 min	640	248 000	−7.44±0.00[c]
	15 min	940	368 000	−7.34±0.17[c]
	20 min	1240	488 000	−7.44±0.00[c]

Means within a column with the same superscripts are not significantly different ($P<0.05$).
[a] All values were means±standard deviation of three values.

temperature decreased by 8 versus 10 °C when pressure was released fast. Concerning pulse series, variations of temperature remained similar during the different pulses applied: there was no cumulative effect of temperature caused by pulse series (Fig. 2).

3.2. Effect of pressurization and depressurization kinetic parameters (one pulse)

The effect of kinetic parameters on the reduction of *S. typhimurium* and *L. monocytogenes* is shown in Tables 2 and 3 respectively. The poor inactivation obtained, lower than 1 log, showed that the application of pressurization and depressurization alone (without a constant pressure stage) did not induce a sharp inactivation of microorganisms. Nevertheless, in both cases the most significant reduction was obtained with the longest treatment, corresponding to 1 MPa/s pressurization and 5 MPa/s depressurization rates. These slow pressurization and depressurization rates provided the highest BP (MPa.s). Then for one pulse the slowest pressurization and depressurization rates promoted the best reduction of microorganisms. We can suppose that for each pressure of treatment, there is a correlation between the BP applied and the reduction of microorganisms. The calculation of the efficiency of the treatment might help us to check this hypothesis: efficiency of treatment was used to compare the effectiveness of the reduction brought back to an identical BP (MPa.s). Tables 2 and 3 show that the same reduction of microorganisms was obtained with the various combinations of pressure applied and processing time. This was true for one level of pressure and both microorganisms. Nevertheless this assumption is true only in the case of treatment applied in the form of a single pulse. Generally food decontamination also includes either holding time or pulse series. It is thus interesting to determine whether the pressurization/depressurization kinetic parameters can modify the efficiency of these two types of treatment used in the food industry.

3.3. Effect of high pressure processing by pulse series

Results of reduction of *S. typhimurium* and *L. monocytogenes* treated by pulse series are presented in Tables 4 and 5 and Fig. 3. Reduction of microorganisms obtained with pulse series was higher when the number of pulse increased. We also demonstrated by a significant statistical difference, that the more the number of pulses increased, the larger was the reduction of *S. typhimurium* and *L. monocytogenes*, whatever the pressurization/depressurization kinetic parameters applied. However, the slowest kinetic parameters applied caused a higher reduction of microorganisms than the fastest parameters. This result can be attributed to the BP applied, as we previously showed. Application of successive pulses induced a linear reduction of microorganisms as shown in Fig. 3. Few authors have studied high pressure application by pulses. López-Caballero et al. (2000) demonstrated on oysters that step-pulse pressurizing (400 MPa at 7 °C in two 5 min pulses) produced no apparent advantage over continuous pressurizing on the reduction of the microbial flora. High pressure pulses have

Table 5
Effects of high pressure processing conditions, pulse series (1, 2 or 3) and various holding times (5, 10, 15 and 20 min) on inactivation of *Listeria monocytogenes* in phosphate buffer pH 7 after pressurization at 500 MPa with different kinetic parameters of pressurization/depressurization

Treatment of *Listeria monocytogenes* at 500 MPa				
		Time of treatment (s)	Barometric power (MPa.s)	Reduction $\log_{10}(N/N_0)$[a]
Slowest kinetic parameters: (pressurization at 1 MPa/depressurization at 5 MPa/s)				
Number of pulses	1	600	150 000	−0.57±0.23[a]
	2	1200	300 000	−1.87±0.36[b]
	3	1800	450 000	−2.79±0.49[c]
Holding time at 500 MPa	5 min	900	300 000	−2.81±0.31[c]
	10 min	1200	450 000	−5.47±0.20[d]
	15 min	1500	600 000	−5.61± 0.53[d]
	20 min	1800	750 000	−5.75±0.42[d]
Fastest kinetic parameters: (pressurization at 10 MPa/quick depressurization < 2 s)				
Number of pulses	1	50	12 500	0.10±0.25[a]
	2	100	25 000	−0.47±0.27[b]
	3	150	37 500	−0.67±0.29[b]
Holding time at 500 MPa	5 min	350	162 500	−5.06±0.11[c]
	10 min	650	312 500	−6.17±0.11[d]
	15 min	950	462 500	−6.34±0.21[d]
	20 min	1250	612 500	−7.17±0.68[e]

Means within a column with the same superscripts are not significantly different ($P<0.05$).
[a] All values were means±standard deviation of three values.

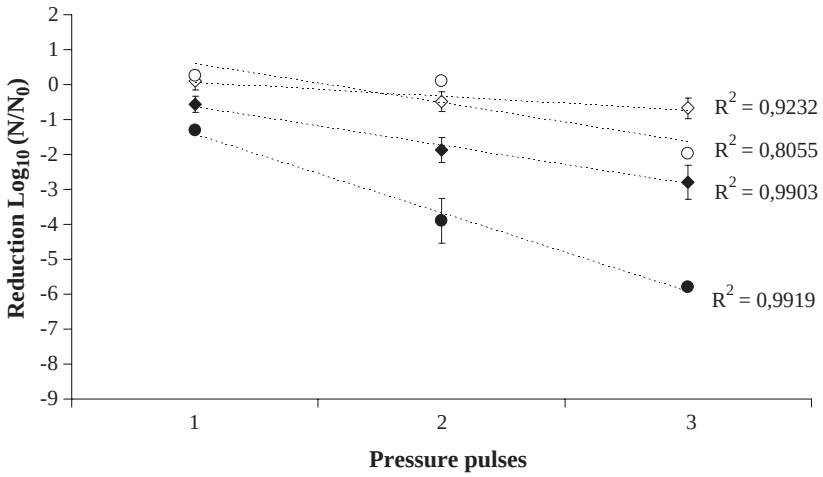

Fig. 3. Effect of pulse series (1, 2 or 3) processing with various pressurization/depressurization kinetic parameters on the reduction of *Salmonella typhimurium* (● and ○) and *Listeria monocytogenes* (♦ and ◊) treated at 400 and 500 MPa respectively. Black symbols (●, ♦) were pressurised at 1 MPa/s then depressurized at 5 MPa/s, white symbols (○, ◊) were pressurized at 10 MPa/s then 250 MPa/s depressurized.

been also successfully used to inactivate pectin methyl esterase (PME) in concentrated orange juices by Basak and Ramaswamy (2001). Mussa et al. (1999) described high pressure inactivation of *L. monocytogenes* by a dual effect which consisted firstly of a step-change in the number of survivors following the application of a pressure pulse with no holding time. This principle was named instantaneous pressure kill effect, the pressure killing value changing according to the microorganism studied. These authors assumed that the instantaneous pressure kill effect was due to rapid pressurization/depressurization cycle. A quick depressurization of compressed microbial cells might cause an adiabatic expansion of the cytoplasmic material that may cause the cell death. There is a discrepancy between our results and those of Mussa as we proved that the application of pulses (from one to three successive) induced a higher inactivation with slow rates of pressurization/depressurization, than with fast rates of pressurization/depressurization.

3.4. Effect of high pressure holding time

Results of reduction of *S. typhimurium* and *L. monocytogenes* treated with various holding times are presented in Tables 4 and 5 and Fig. 4. The microbial survival was highly influenced by both the holding time and kinetic parameters of treatment. Higher holding times were more effective in the destruction of *S. typhimurium* and *L. monocytogenes* cells. For both organisms the results show that the best reduction was obtained with the fastest kinetic parameters combined with pressure holding time. These results confirm those of Noma et al. (2002) who demonstrated that fast depressurization of the vessel induced a higher reduction of vegetative cells. This suggests that the fastest pressurization/depressurization procedure could enhance the degree of pressure-mediated injury induced by a more significant stress on microorganisms and cause the bactericidal effect. Concerning *S. typhimurium*, whatever the pressurization/depressurization kinetic para-

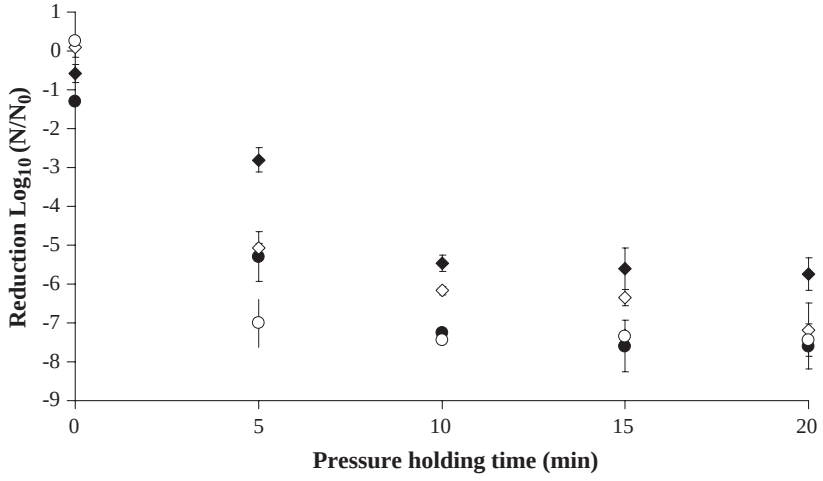

Fig. 4. Effect of holding time (5, 10, 15 or 20 min) processing with various pressurization/depressurization kinetic parameters on the reduction of *Salmonella typhimurium* (● and ○) and *Listeria monocytogenes* (♦ and ◊) treated at 400 and 500 MPa, respectively. Black symbols (●, ♦) were pressurized at 1 MPa/s then depressurized at 5 MPa/s, white symbols (○, ◊) were pressurized at 10 MPa/s then 250 MPa/s depressurized.

meters, a treatment at 400 MPa during 10 min induced a total inactivation of cells. As regards *L. monocytogenes*, the influence of pressurization/depressurization kinetic parameters was more sure because 500 MPa for 20 min (the longer holding time) were necessary to obtain the total destruction of cells. These results confirm the greatest pressure resistance of Gram positive bacteria (Hoover, 1993). Fig. 4 shows a non-linear reduction of the microorganisms according to holding time. Curves of reductions obtained are logarithmic and characterize the resistance of microorganisms.

3.5. Comparison between high pressure treatment procedures

Comparison of the treatments applied to reduce *S. typhimurium* and *L. monocytogenes* showed that holding time was more efficient than pulse series. Treatment by pulse series of *L. monocytogenes* induced a maximum reduction of -2.79 ± 0.49 after 3 pulses with the lowest kinetics parameters and corresponding to a total duration of treatment of 30 min, while a similar result was obtained with a 5 min holding time (-2.81 ± 0.31) under the same conditions of pressurization/depressurization. Values of the same order of reduction were obtained with *S. typhimurium*, reduction of -5.79 ± 0.03 with 3 pulses and -5.29 ± 0.67 with a 5 min holding time. With the fastest kinetic parameters of pressurization/depressurization, the reduction induced by holding time was definitely more significant than pulse series. Indeed in this case, the BP provided by pulses was much lower than that induced by the holding time (Tables 4 and 5).

4. Conclusions

Our experiments showed that the reduction obtained for a target value of pressure of 400 MPa for *S. typhimurium* and 500 MPa for *L. monocytogenes*, respectively, depends on the BP applied as expressed by the pressure–time product (MPa.s). In the case of treatment by a single pressure pulse, results revealed that the slowest pressurization/depressurization kinetic parameters led to the most significant reduction of microorganisms.

As regards industrial applications, food product decontamination by holding time treatment brings about a more significant reduction than treatment by pulse series. Furthermore, combining pressure holding time with the fastest pressurization/depressurization kinetic parameters induced a higher efficiency of the treatment. Holding of pressure for a given time induced a bacterial stress, and when combined with the fastest pressurization/depressurization kinetic parameters it improved the reduction of the microorganisms.

We also observed a linear reduction of microorganisms with the number of pulses, and a logarithmic reduction with the holding time, which reveals a previously undescribed resistance form. Combining both treatments, for example several short holding times applied successively, could be explored in order to improve the effect of high pressure on food bacterial decontamination.

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