The effect of growth temperature, process temperature, and sodium chloride on the high pressure inactivation of *Listeria monocytogenes* on ham

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Abstract

This study investigated the effect of growth temperature (8 to 32°C), process temperature (-17 to 32°C), and sodium chloride concentration (0 to 3%) on the lethality of pressure to *Listeria monocytogenes*. Pressure treatments were performed using a 5-strain cocktail of *L. monocytogenes*. Cultures grown at 8ºC were more resistant to pressure than cultures grown at 20 or 32°C. Pressure treatments of the *Listeria* cocktail indicated that *Listeria* were most resistant to pressure at -5ºC or +5ºC. The effect of pressure was further evaluated at 500 MPa and +5ºC in buffer containing 1 or 3% NaCl. Cultures treated in the presence of 3% NaCl were more resistant than cultures treated in presence of 1% NaCl. Results obtained in buffer were compared to treatment of cooked ham containing 1 or 3% NaCl. *L. monocytogenes* was more resistant in ham with 3% NaCl when compared to ham with 1% NaCl. *L. monocytogenes* grown at 32°C were slightly more resistant to pressure when compared to cultures grown at 8°C. Refrigerated storage of treated samples for four weeks demonstrated that *L. monocytogenes* recovered from all treatments with a pressure holding time of 8 min or less. In conclusion, the effect of high pressure processing strongly depends on growth temperature, process temperature, and the food matrix. To generally achieve a 5 log reduction of *L. monocytogenes* on ready-to-eat meats, combinations of pressure with elevated temperature or other antimicrobial hurdles are necessary.

Key words: High pressure processing, Inactivation, *Listeria monocytogenes*, Growth temperature, Treatment temperature, Ready-to-eat meat.

41 **Introduction**

42 In North America, government policies encourage the use of pressure to eliminate *Listeria*
43 *monocytogenes* on ready-to-eat (RTE) meats. Current industry practice employs 600 MPa for 3 –
44 5 min in refrigerated processing plants [1]. Process temperature and pressure impact the
45 inactivation of *L. monocytogenes* [2, 3, 4, 5, 6]; however, current industrial practice often does
46 not provide adequate control of the temperature during pressure treatment. A process
47 temperature below 10°C or above 35°C increased pressure sensitivity of *Listeria* when compared
48 to treatments at ambient temperature [3, 4]. Stationary-phase cells are more resistant to pressure
49 than exponential-phase cells [7, 8]. The growth temperature also strongly influences the
50 bactericidal effects of pressure treatment. Cultures grown to stationary phase near the optimum
51 growth temperature of 37°C are more resistant to pressure than stationary phase cultures grown
52 at low temperatures [5, 7, 8], matching previous observations on the effect of the growth
53 temperature on the pressure resistance of *Lactobacillus plantarum* [9]. However, a cold shock of
54 exponentially growing cells increased the resistance of *L. monocytogenes* to subsequent
55 treatment at 300 MPa 100-fold [10]. These results suggest that cold adaptation of *L.*
56 *monocytogenes* may provide cross-protection against pressure.

57 RTE meats are typically contaminated with *L. monocytogenes* during slicing and packing, and
58 contaminating cells have grown in a refrigerated processing environment [11]. Cold adaptation
59 of *L. monocytogenes* involves adjustment of membrane fluidity, overexpression of cold shock
60 proteins, and the accumulation of compatible solutes [10, 12, 13, 14]. An increase of the
61 membrane fluidity in response to reduced growth temperature reduced the resistance of *Lb.*
62 *plantarum* to pressure, and accelerated the inactivation of integral membrane proteins [9]. In
63 contrast, a 3.5 fold overexpression of cold shock proteins in response to a temperature downshift

64 was related to an increased pressure resistance of *L. monocytogenes* [10]. The accumulation of
65 the compatible solutes glycine betaine and carnitine in response to low growth temperatures, or
66 in response to elevated osmolarity of the growth medium, increases pressure resistance of *L.*
67 *monocytogenes* [14]. Baroprotective mechanisms of compatible solutes relate to the stabilization
68 of proteins, membrane, and ribosomes based on the principle of preferential hydration [13].
69 Cold adaptation of *Listeria* includes physiological changes that increase the resistance to
70 pressure, and mechanisms of cold adaptation are intricately linked to osmoprotection. Changes in
71 the NaCl content of RTE meats or the increase of the osmotic pressure by addition of
72 preservatives may thus have an impact on the survival of *L. monocytogenes* during pressure
73 treatment. However, currently available literature data provides little information on the effect of
74 the growth temperature on the pressure resistance of *L. monocytogenes* on RTE meats and salt-
75 reduced RTE meats. This study aimed to investigate the effect of growth temperature, process
76 temperature, and sodium chloride concentration on the pressure resistance of *L. monocytogenes*.
77 Pressure treatments were carried out in laboratory media or in ham containing 1 or 3% NaCl.

78 **Material and Methods**

79 Bacterial strains and growth conditions.

80 The five strains of *L. monocytogenes* used in this study, strains FSL J1-177, FSL C1-056, FSL
81 N3-013, FSL R2-499, and FSL N1-227, form the "human disease cocktail" recommended for
82 challenge studies in food [15]. For the preparation of working cultures, strains were streaked
83 from -80°C stock cultures onto Tryptic Soy (TS) agar (Difco, Becton Dickinson, Sparks, MD,
84 USA), followed by inoculation into TS broth (TSB) and incubation overnight at 20°C. Fresh
85 broth was inoculated with 1% (v/v) of the overnight culture and incubated at 8, 20 or 32°C to the
86 stationary growth phase. To exclude the influence of the growth phase on pressure resistance,

87 growth curves of the five different strains were obtained for each growth temperature and aligned
88 by re-scaling the time axis (Figure 1). Cultures for use in pressure experiments were harvested
89 after 156, 20, and 16 h of growth at 8, 20, and 32°C, respectively, to obtain cultures that were
90 grown to equivalent points of the growth curves.
91 For preparation of cocktails, an equal volume of each individual culture was mixed to form a 5-
92 strain cocktail of *L. monocytogenes*. This cocktail was harvested by centrifugation (7000 × *g* for
93 5 min) and resuspended in an equal volume of TSB without or with addition of 1 or 3% (w/v)
94 NaCl. For experiments with ham, the cocktail was resuspended in an equal volume of saline
95 solution (0.85% NaCl).
96 Preparation of ham.
97 Cooked ham with a final sodium chloride concentration of 1% (w/w) or 3% (w/w) was prepared
98 according Liu et al. [16]. The formula of ham consisted of ground pork, ice (20% of meat
99 weight), sodium tripolyphosphate (0.63% of meat weight), sodium erythorbate (0.1% of meat
100 weight), dextrose (3.1% of meat weight), Prague powder containing 6% $NaNO_2$ and 94% NaCl
101 (0.46% of meat weight), and NaCl (0.82 or 3.36% of meat weight for 1 or 3% NaCl in the final
102 product). After production and cooling, the hams were cut to 20 mm thick slices with a surface
103 area of 50 cm^2, vacuum packaged, and stored at 0°C until use.
104 High pressure equipment.
105 The high pressure system (Micro-system, Unipress, Warsaw, Poland) consisted of a high-
106 pressure micropump MP5, a high-pressure autoclave MA1, and an external control unit. The
107 inner vessel volume was 2.2 ml with a 9 mm inner diameter and 24 mm depth. Bis (2-ethylhexyl)
108 sebacate (Sigma-Aldrich, Germany) served as pressure-transmitting medium. The vessel was
109 submerged in a water bath (Isotemp, Fisher Scientific, USA) filled with a glycol-water mixture

and the internal temperature was measured by an integrated type K thermocouple positioned inside the vessel. Compression and decompression rates were 277.8 MPa/min. Figure 2 shows the development of the temperature during pressure treatments. Immersion of the pressure vessel in the water bath limited the temperature increase in the pressure vessel due to compression heating to less than 5°C.

To mimic industrial settings, experiments at 600 MPa were performed with pressure equipment with a working volume of 300 mL (Dustec High Pressure 10,000 Bar, Germany). Glycerol served as pressure-transmitting medium and the process temperature was maintained by a thermostat jacket connected to a water bath. Compression and decompression rates were 100 MPa/min.

Pressure inactivation of *L. monocytogenes* in broth.

Aliquots of 65 µL of *L. monocytogenes* FSL J1-177 or the 5-strain cocktail of *L. monocytogenes* were transferred to Tygon tubing (Tygon S3™ E-3603 Flexible Tubings, Fisherbrand™, Pittsburgh, USA) and heat-sealed [17]. Samples were maintained at the temperature of growth until pressure treatment. After placement of samples in the pressure vessel, the temperature was equilibrated to the treatment temperature for 10 minutes prior to compression.

Treatment conditions for *L. monocytogenes* FSL J1-177 were as follows: Growth temperature of 8, 20, or 32°C, each treated at 500 MPa at -17, -5, +5, +15, and +32°C for 0 to 32 min.

Treatment conditions for the 5-strain cocktail of *L. monocytogenes* were as follows: Growth temperature of 8, 20, or 32°C, each treated at 500 MPa at -5 and +5ºC for 0 to 32 min. Treatments were also performed at 500 MPa at +5ºC for 0 to 32 min in TSB adjusted to 1 or 3% (w/v) NaCl.

132 Detection of surviving cells was determined by surface plating as described below. Experiments
133 were performed in triplicate independent experiments.
134 Pressure inactivation of *L. monocytogenes* on ham.
135 Ham slices containing 1% or 3% NaCl were aseptically removed from vacuum packages, and
136 cylindrical ham samples were shaped with a sterile cork borer (Fisher Scientific, USA). To
137 achieve an initial cell count of 10^7 colony forming units (CFU), ham cylinders were dipped three
138 times for approximately 15 seconds into the 5-strain cocktail preparation with saline solution
139 (0.85% NaCl). The samples were transferred aseptically into Tygon tubing and heat-sealed.
140 Samples were maintained at the temperature of growth until pressure treatment. After placement
141 of the samples in the pressure vessel, the temperature was equilibrated for 10 minutes prior to
142 compression. Ham samples were treated at 500 MPa at -5, +5 and +32°C for 0 to 32 min.
143 Detection of surviving cells was determined by surface plating as described below. Additionally,
144 separate sets of treated samples were stored for 4 weeks at 4°C after treatment, and the presence
145 of viable cells was detected as outlined below.
146 Ham slices containing 1% Na were surface-inoculated with the 5-strain cocktail in saline
147 suspension to achieve a final concentration of 10^6 CFU/cm^2. Ham slices were treated at 600 MPa
148 at 5 or 20°C for 0 to 30 min under conditions simulating the meat industry practice. Surviving
149 cells were enumerated by surface plating as outlined below.
150 Detection of surviving cells
151 The presence or absence of *L. monocytogenes* was monitored immediately after pressure
152 treatment, and after storage for 4 weeks at 4°C. Non-treated samples were prepared to determine
153 the initial cell count. Samples were opened aseptically, the contents transferred to a sterile 1.5
154 mL Eppendorf tube, and diluted with sterile saline (0.85% NaCl). Ham samples were

155 homogenized for 60 seconds prior to serial dilutions. Surviving cells were enumerated by plating
156 on selective PALCAM agar (Becton Dickinson) and non-selective TS agar (Difco) for
157 determination of viable and sublethally injured cells. Appropriate dilutions were plated and
158 incubated at 37°C for 48 hours. The inactivation results were expressed as the relative survivor
159 fraction $\log_{10}$ (N/N_0), where N is the number of CFU after treatment and N_0 is the number of
160 CFU of the untreated bacteria, which was determined for each experiment.
161 Statistical analysis
162 All experiments were replicated at least three times. Data were converted to log reductions
163 [$\log(N/N_0)$ where N represents the cell count of treated samples and N_0 represents the cell count
164 of untreated samples] and subjected to analysis of variance using the PROC GLM procedure of
165 SAS University Edition (Version 3.4; SAS Institute. Inc., Cary, NC, USA). Differences among
166 means were determined using Student Newman Kuel's multiple range test at an error probability
167 of 5% ($P < 0.05$).
168 **Results**
169 Impact of growth and process temperatures on process lethality
170 The interaction of growth temperature and process temperature on the lethality of pressure
171 treatment was initially determined with a single strain, *L. monocytogenes* FSL J1-177 grown at 8,
172 20, or 32°C in TSB and treated at 500 MPa and -17, -5, +5, +15 or +32°C (Figure 3). Cells
173 grown at 8ºC were more resistant than cells grown at 20 or 32ºC (Figure 3). The treatment time
174 needed to achieve a reduction of 5 log (CFU/mL) was 4 min or more for cultures grown at 8°C
175 but 2 min or less for cultures grown at 20 or 32°C. *L. monocytogenes* was most resistant to
176 pressure treatment at -5ºC.

177 Treatments at 500 MPa and -5ºC or +5ºC for 0 to 32 min were also performed with a 5-strain
178 cocktail of *L. monocytogenes* (Figure 4). Results from the treatment of the strain cocktail
179 conformed to results obtained with *L. monocytogenes* FSL J1-177. Cells grown at 8ºC were more
180 resistant to pressure than cells grown at 20 or 32ºC. Cells of the 5-strain cocktail of *L.*
181 *monocytogenes* that were grown at 20 or 32°C were most resistant to pressure treatments at
182 +5ºC. Surviving cells were detected after every pressure treatment when cell counts were
183 enumerated on TS agar (Figure 4). However, cell counts on PALCAM were reduced below 2 log
184 (CFU/mL) after 8 min of treatment or less (8°C cultures), 3 min or less (20°C cultures) and 2
185 min or less (32°C cultures), indicating that surviving cells were sublethally injured.
186 Impact of growth temperature and salt content on process lethality
187 The effect of pressure was further evaluated by treatments at 500 MPa at +5ºC for 0 to 32 min
188 with TS broth containing 1 or 3% (w/v) NaCl (Figure 5). Cultures grown at 8ºC were most
189 resistant to pressure. Cultures treated in the presence of 3% NaCl appeared to be more resistant
190 than cultures treated in the presence of 1% NaCl, but the influence of NaCl was not observed for
191 cultures grown at 8°C and less pronounced than the influence of the growth temperature.
192 Survivor curves obtained by plating onto TS agar exhibited tailing but all cell counts on
193 PALCAM were reduced below 2 log (CFU/g) after 8 min of treatment (Figure 5B).
194 Impact of growth temperature and salt content on process lethality on ham
195 Treatments in TS broth indicated that *L. monocytogenes* was most resistant to pressure when
196 grown below the optimum temperature, and when treated in the presence of 3% NaCl. These
197 results were verified by treatment on ham with two salt concentrations. To detect recovery of
198 pressure-injured cells, *L. monocytogenes* was enumerated immediately after pressure treatment,
199 and after four weeks of storage at 4°C.

200 The inactivation of *L. monocytogenes* on ham is shown in Figure 6. Consistent with the
201 treatments in broth, *L. monocytogenes* was more resistant to pressure on ham containing 3%
202 NaCl than on ham with 1% NaCl. In contrast to treatments in broth, cultures grown at 8°C were
203 significantly more sensitive than cultures grown at 32°C; interactions of growth temperature and
204 processing temperature on the reduction of cell counts were not significant (Figure 6). The effect
205 of the processing temperature on survival and sublethal injury of *L. monocytogenes* was
206 significant but relatively minor (Figure 6).
207 Although treatment at 500 MPa reduced cell counts of *L. monocytogenes* below the detection
208 limit after 4 – 16 min of treatment, sublethally injured cells recovered after 4 weeks of storage.
209 Recovery and growth were detected in more than 80% of the samples (Figure 6). None of the
210 pressure treatments prevented cell recovery and growth at a pressure holding time up to 8 min.
211 At a pressure holding time up to 16 min, only one sample treated at 32°C remained *Listeria*-
212 negative after 4 weeks of storage. Even pressure treatments for 32 min did not prevent cell
213 recovery in all samples (Figure 6). Differences between the lethality of treatments at a process
214 temperature 5°C and treatments at 32 or -5 °C were not significant or inconsistent; however,
215 *L. monocytogenes* was more resistant to pressure treatment in ham at -5°C when compared to a
216 process temperature of 32°C.
217 To determine the survival of *L. monocytogenes* on ham at the pressure level that is currently
218 employed in industrial practice, ham containing 1% NaCl was treated at 600 MPa and 5°C or
219 20°C (Figure 7). The reduction of cell counts after treatment at 600 MPa was faster when
220 compared to treatments at 500 MPa; however, surviving cells were detected after all treatments
221 and the process lethality did not exceed 4 log (CFU/cm^2) even if the pressure holding time
222 exceeded 5 min (Figure 7).

Discussion

This study evaluated the combined effects of growth and pressure processing temperatures on the viability of *L. monocytogenes* after treatment in broth or on ham. We also monitored recovery of *L. monocytogenes* after 4 weeks of post-pressure storage at 4°C. Our results confirmed prior observations that an increase of the process temperature increases the process lethality on *L. monocytogenes* [2, 5, 6]. However, treatment at sub-zero temperatures did not increase the process lethality and *L. monocytogenes* was most resistant to treatment at a process temperature of -5°C. Phase transitions between ice I and ice III may reduce the cell counts of *L. monocytogenes* [18].

We obtained conflicting results for the effect of the growth temperature on pressure resistance of *L. monocytogenes*. Careful standardization of the growth curves at different temperatures excluded an influence of the growth phase as a confounding factor (Figure 1). Cultures grown at 8°C were most resistant to pressure treatment in TS broth but least resistant to pressure treatment on ham. These results partially contrast previous data indicating that cultures grown near the optimum temperature of growth are most resistant to pressure [5, 7, 8], and emphasize that mechanisms of cold adaptation and adaptation to osmotic stress are interrelated.

In response to a sudden temperature downshift, or in response to low growth temperatures, *L. monocytogenes* increases the expression of cold shock proteins [19]. These proteins are related to a large family of nucleic acid binding proteins [20]. It is assumed that they bind RNA and DNA, support metabolic functions such as replication, transcription and translation, and thus maintain cellular function under cold stress [20]. Their contribution to pressure resistance remains unknown.

245 Cold adaptation of *L. monocytogenes* also includes adjustment of the membrane composition to
246 maintain the membrane in the liquid crystalline phase. Membrane phospholipids of
247 *L. monocytogenes* are characterized by a high proportion of iso and anteiso, odd-numbered,
248 branched-chain fatty acids [11, 21]. The proportion of anteiso $C_{15:0}$ fatty acids is increased at the
249 expense of iso $C_{15:0}$ and $C_{17:0}$ fatty acids during cold adaptation of *L. monocytogenes* [21].
250 Furthermore, the proportion of unsaturated fatty acids in membrane fatty acids is increased [22].
251 An increase in membrane fluidity in response to low growth temperature was suggested to
252 decrease pressure resistance of *Lb. plantarum* [9]. However, our study included temperature
253 equilibration for 10 min prior to compression to adjust the sample temperature from the
254 temperature of growth to the treatment temperature. Prior data on the kinetics of the response of
255 the membrane fluidity to temperature changes [12] indicate that 10 min of equilibration suffice to
256 adjust the composition of membrane lipids, and hence membrane fluidity. *L. monocytogenes*
257 strains Scott A, NR30, wt10304S and cld1 adjusted their membrane composition to the altered
258 ambient temperature 130 to 180 seconds after a temperature upshift from 15°C to 30°C [12]. The
259 rapid adjustment of the membrane fluidity in response to temperature changes prior to
260 compression may partially obscure the effect of the growth temperature on pressure resistance.
261 Bacteria accumulate compatible solutes in response to low growth temperature, or in response to
262 increased ambient osmolarity [14, 23]. Compatible solutes can be accumulated to high
263 intracellular concentrations without affecting cytoplasmic functions [24]. Glycine betaine and
264 carnitine are the major cryoprotectant and osmoprotectant compatible solutes in
265 *L. monocytogenes*. The osmolyte transporters glycine betaine transporter I (BetL), glycine
266 betaine transporter II (Gbu) and carnitine transporter OpuC mediate their uptake [23, 25, 26].
267 Comparable to the homeoviscous adaptation of the membrane in response to changes in the

ambient temperature, the uptake or expulsion of compatible solutes after changes in the ambient osmotic pressure is rapid and occurs within milliseconds [27].

Accumulation of compatible solutes increases the bacterial resistance to pressure [14, 28]. Transfer of *Listeria* from TS broth to TS broth with higher osmolarity, or onto ham likely induced uptake of compatible solutes prior to compression. This uptake may have equilibrated any differences in the intracellular concentration of compatible solutes that resulted from the different growth temperatures [23]. Prior studies on the role of compatible solutes on bacterial pressure resistance employed more than 3% NaCl [14, 28]. It is thus remarkable that the modest decrease of the NaCl content of ham from 3% to 1% had a measurable impact on pressure resistance of *L. monocytogenes* (Figures 4, 5, and 6). The meat industry currently aims to reduce the salt content of ready-to-eat meat products [29]. The present study indicates that even a modest reduction of the NaCl content can improve food safety by decreasing the pressure resistance of *L. monocytogenes*.

Treatment of ham at 500 or 600 MPa reduced cell counts of *L. monocytogenes* by maximum of 4 log (CFU/cm^2); however, survivor curves exhibited a strong tailing effect. This was consistent with prior studies on the pressure resistance of *L. monocytogenes* [30, 31, 32, 33]. As a consequence of these strong tailing effects, virtually all ham samples analysed in this study tested positive for *L. monocytogenes* after treatments at 500 MPa or 600 MPa followed by 4 weeks of refrigerated storage. In Canada, pressure processing is used to increase the safety and storage life of ready-to-eat meats but cannot be used to establish the safety of ready-to-eat meats that tested positive for *L. monocytogenes* [1]. The limited listericidal effect of treatment as per current industrial practice (600 MPa for 3 – 9 min) supports this policy.

290 In conclusion, the present study demonstrates that the listericidal effect of pressure strongly
291 depends on process temperature and the food matrix. The high osmolarity of ready-to-eat meat
292 products contributes to the protective effect of the food matrix; remarkably, a reduction of the
293 NaCl content from 3 to 1% decreased the resistance of *L. monocytogenes* to pressure. When used
294 at ambient process temperature, pressure processing does not eliminate the risk associated with
295 *L. monocytogenes* unless other pathogen interventions are in place, particularly good
296 manufacturing practice to prevent contamination during slicing and packaging. Further
297 exploration of the p/T landscape or combined use with antimicrobial agents is necessary to
298 increase the bactericidal effect of current industrial processes for meat preservation.

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Figure 1. Growth curves for the individual *L. monocytogenes* strains J1-117, C1-056, N3-013, R2-499 and N1-227 in Tryptic Soy Broth (TSB) at 8 (white), 20 (grey), and 32°C (black). Data are shown as means ± standard deviations of triplicate independent experiments. The x-axes are offset to overlap the growth curves of cultures that were incubated at different temperatures.

Figure 2. Temperature and pressure profile over time. The dashed line shows the pressure trajectory as a function of time with a maximum pressure of 500 MPa. The solid lines represent the temperature profiles over time and pressure.

Figure 3. Survivor curves for *L. monocytogenes* (FSL J1-177) following high-pressure treatment at 500 MPa at -17, -5, +5, +15, and +32°C for 0 to 32 min. *L. monocytogenes* was grown to the stationary phase in TSB at 8 (white), 20 (grey), and 32°C (black). N_0, initial number; N, number of survivors. Cell counts of untreated samples are shown with a pressure holding time of -1 min; samples subjected to compression, followed by immediate decompression are shown with a pressure holding time of 0 min. Data are shown as means ± standard deviations of triplicate independent experiments.

Figure 4. Cell counts of a *L. monocytogenes* strain cocktail following high-pressure treatment at 500 MPa at -5 (black) and +5°C (white) for 0 to 32 min. *L. monocytogenes* strains were grown in TSB at 8 (●); 20 (▼); 32°C (■). N_0, initial number; N, number of survivors. Cell counts of untreated samples are shown with a pressure holding time of -1 min; Samples subjected to compression, followed by immediate decompression are shown with a pressure holding time of 0 min. Data are shown as means ± standard deviations of triplicate independent experiments.

Figure 5. Cell counts of a *L. monocytogenes* strain cocktail following high-pressure treatment at 500 MPa at +5°C for 0 to 32 min. Cell counts were enumerated on TSA (A) and PALCAM (B). *L. monocytogenes* strains were grown at 8 (●); 20 (▼); 32°C (■) in TSB containing 1% NaCl

(white) or 3% NaCl (black). N_0, initial number; N, number of survivors. Cell counts of untreated samples are shown with a pressure holding time of -1 min; samples subjected to compression, followed by immediate decompression are shown with a pressure holding time of 0 min. Data are shown as means ± standard deviations of triplicate independent experiments.

Figure 6. Effect of NaCl and different growth temperatures on the inactivation of *L. monocytogenes* at 500 MPa at -5 (A, D); +5 (B, E); +32°C (C, F) for 0 to 32 min on ham. Cell counts were enumerated on TSA (A, B, C) and PALCAM (D, E, F). Cells were cultured at 8 (black) and 32°C (white). Ham contained 1% NaCl (●) or 3% NaCl (▼). The table illustrates the number of samples showing cell recovery for 8, 16 and 32 min of pressure holding time of samples treated with high-pressure at +32 (A); +5 (B); -5°C (C) and stored for 4 weeks at 4°C. Cell counts of untreated samples are shown with a pressure holding time of -1 min; samples subjected to compression, followed by immediate decompression are shown with a pressure holding time of 0 min. The interception point of abscissa and ordinate represents the highest detection limit.

Data are are shown as means ± standard deviations of triplicate independent experiments and differences among means were determined using Student Newman Kuel's multiple range test with $P < 0.05$. Significant differences are indicated as follows:

*, treatments on ham with 3% NaCl were less lethal than treatments on ham with 1% NaCl;

#, treatments at -5°C were less lethal than treatments at 32°C;

+, treatments of cultures grown at 32°C were less lethal than treatments of cultures grown at 8°C.

Figure 7. Reduction of cell counts of a *L. monocytogenes* strain cocktail following high-pressure treatment at 600 MPa at +5°C (black) and 20°C (grey) for 0 to 10 min on ham. Samples were inculated to 6 ± 0.2 log(cfu/cm^2) and cell counts of treated and control samples were enumerated

on TSA. Ham contained 1 % NaCl. Samples subjected to compression, followed by immediate decompression are shown with a pressure holding time of 0 min. Data are shown as means ± standard deviations of triplicate independent experiments.