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EFFECT OF HIGH HYDROSTATIC PRESSURE AND NISIN ON MICRO-ORGANISMS IN MINCED MEATS

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The effect of high hydrostatic pressure (HHP) and nisin was studied on micro-organisms in minced chicken and beef meat. Pressure in the range of 0–800 MPa and nisin (670 IU g^{-1}) were applied for vacuum packed minced meat. In chicken meat the total viable cell count decreased by 3 log cycles as an effect of HHP at 300 MPa and by 5 log cycles in combination with nisin. The D value is 35–39 MPa for pseudomonads in minced chicken meat. In case of inoculation with *L. monocytogenes*, the cell count in beef meat was reduced only by pressure higher than 200 MPa ("shoulder") with a characteristic value of D=37–38 MPa. *B. cereus* spores, both dormant and heat activated, were very resistant (D=800 MPa) in beef. However, the survival of pressurised spores after chilled storage (for two weeks at 4 °C) was smaller for non-heat activated spores than for heat activated spores. Efficiency of HHP combined with nisin needs further research work.

Keywords: high hydrostatic pressure, nisin, combined treatment, decontamination

It is well known that vegetative micro-organisms are sensitive to high hydrostatic pressure (HHP), therefore this process is an effective treatment to inactivate or kill spoilage and pathogenic microbes from food safety point of view (HOOVER et al. 1989; KNORR, 1993; SMELT, 1998). One approach to improve the effect of preservation methods is to combine the treatments by reducing the sensitivity of micro-organisms (PATTERSON & KILPATRICK, 1998; CAPELLAS et al., 2000). Our aim was to study the effect of high hydrostatic pressure alone and in combination with nisin on *Listeria monocytogenes,* pseudomonads and total viable cell count of meat and to investigate how nisin increases the effect of hydrostatic pressure on spores of *Bacillus cereus* in minced meat.

1. Materials and methods

Deboned chicken meat with natural microbial contamination was minced and vacuum-packed in WIPAK multibarrier pouches (PE-PA-PE).

Beef meat (musculus psoas maior) was used 24 h after slaughter. The microbial load (total viable cell count) was 10^3–10^4 CFU g^{-1}. Minced meat was prepared and one

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part of it was inoculated with a suspension of an overnight culture of *Listeria monocytogenes* strain OÉTI 493 KR (10^6–10^7 CFU g^{-1} meat). The other part was inoculated with a stock suspension of dormant spores of *Bacillus cereus* (psychrotrophic strain, F 46.29.90 ATO-DLO, Wageningen). In the first experiment the suspension was heat treated at 60 °C for 10 min to eliminate the vegetative cells prior to mixing it with the meat (spore count 10^6–10^7 CFU g^{-1} meat). In the second experiment the spore suspension was heat activated at 80 °C for 10 min and mixed with minced meat, resulting in a spore count of 10^7 CFU g^{-1}.

Nisaplin (Aplin & Barrett Ltd., U.K., 1.0×10^6 IU nisin g^{-1}) was solved in 50% ethanol, centrifuged (MLW T-24) for 2 min at 15×10^3 r.p.m., filtered by MILLEX®-GV 0.22 μm Filter Unit (MILLIPORE), stored for 24 h at 4 °C before use and mixed into meat in the concentration of 670 IU g^{-1}.

In case of naturally contaminated chicken minced meat and minced beef meat inoculated with *L. monocytogenes*, samples between nisin addition and HHP treatment were kept at 20–23 °C or 4 °C for maximum 60 min. Other samples (inoculated with *B. cereus* spores) were stored at 4 °C for maximum time prior to pressure treatment.

The high pressure treatment was carried out with the FOOD LAB 900 equipment (Stansted Fluid Power Ltd., U.K.) in the range of 100–800 MPa. The pressurization time was 20 min and the temperature during treatments was between 0 and 40 °C.

Samples were stored at 4 °C. For microbiological investigations a 10 g sample was homogenised in 90 ml of peptone-sodium chloride solution by a BagMixer 400 VW (INTERSCIENCE) for 1 min and a decimal dilution series was prepared. For colony counting Spiral Plate Technique and for low cell counts MPN method was used. The viable cell counts on Nutrient Agar (MERCK), pseudomonads counts on Pseudomonas Selective Agar or Broth (OXOID) were determined at 30 °C after 48 h. The colony count of *L. monocytogenes* was determined on PALCAM and NUTRIENT Agar or Broth (MERCK) with incubation at 30 °C for 48 h, spore count of *B. cereus* on *B. cereus* Selective Agar (MERCK) with incubation at 30 °C for 24 h.

Statistical description and evaluation (average, standard deviation, regression and curve fitting) were carried out, where sufficient data was available.

2. Results and discussion

2.1. Effect of treatments on total cell count and pseudomonads

The total viable cell count in minced poultry meat decreased by 1 log cycle at 200 MPa and this reduction was greater than 3 log cycles at 300 MPa. In the presence of nisin, this reduction was further increased by about 1.5 log cycle. Regression equations were calculated from the linear part of the survival curves in the pressure range of 100–300 MPa. The sensitivity of pseudomonads is high to hydrostatic pressure. The reduction was 5 log cycles at 300 MPa, and nisin did not significantly influence this effect (Fig. 1).

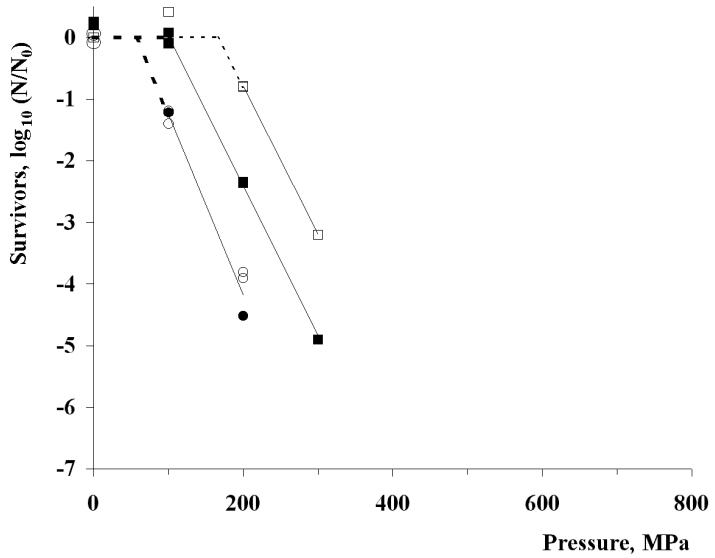

Fig. 1. Survivors of total viable cell count (TVC) and pseudomonads (Ps) in minced chicken meat and the equations of the linear phase as a function of high hydrostatic pressure (p) and nisin

○: Pseudomonads in meat without nisin;	$\log_{10}(N/N_0) = 1.7–0.0293 \cdot p$; $n = 8$
●: pseudomonads in meat with nisin;	(with and without nisin)
□: total viable cell count in meat without nisin;	$\log_{10}(N/N_0) = 4.0–0.0241 \cdot p$; $n = 3$
■: total viable cell count in meat with nisin;	$\log_{10}(N/N_0) = 2.5–0.0243 \cdot p$; $n = 5$

The behaviour of pseudomonads and total viable cell count in our experiments was very similar to that of found by SHIGEHISA and co-workers (1991), CARLEZ and co-workers (1993; 1994). Gram-negative micro-organisms are more sensitive to pressure than Gram-positive ones, therefore high pressure treatment is very effective in reducing the number of pseudomonads causing spoilage.

2.2. Effect of treatments on L. monocytogenes

The effect of high pressure treatment was studied in the 0–500 MPa range in minced beef. At pressures higher than 200–250 MPa, the viable cell count dropped very quickly and at 450 MPa the decrease amounted to more than 6 log cycles. When pressure was applied (0–250 MPa) in the presence of nisin (670 IU g^{-1}), the reduction depended on the temperature of storage between nisin addition and pressure treatment. There was no difference in the survival of viable cells between treated samples stored at 4 °C, while in nisin-containing samples stored at 24 °C the total cell count was 0.5–1.0 log cycle lower compared to the other samples (Fig. 2). This temperature dependent difference was not observed at pressure values higher than 250 MPa. At chilling temperature, the

reduction amounted to 6 log cycles. The slopes of regression equations between 200–450 MPa were statistically not different and the D value with or without nisin was 37 MPa and 38 MPa, respectively (Fig. 3).

Other authors (STYLES et al., 1991; LANCIOTTI et al., 1996) found that the media (milk, meat) have protective effect on micro-organisms and HHP sensitises the micro-organisms to nisin.

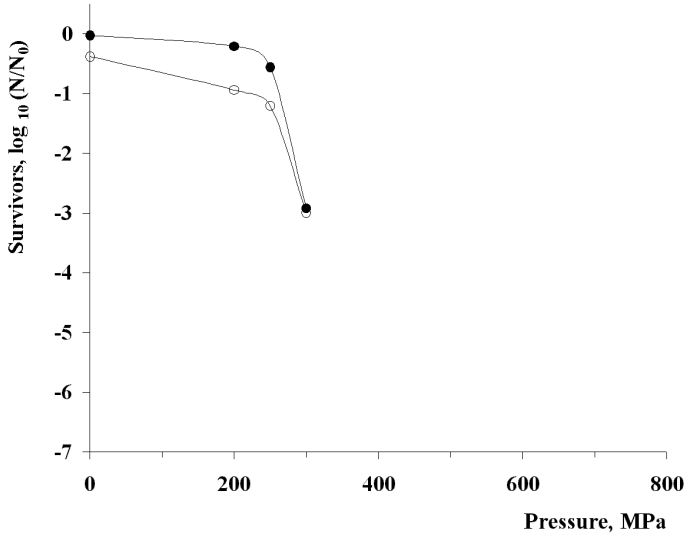

Fig. 2. Survivors of *Listeria monocytogenes* in minced beef as a function of high hydrostatic pressure and nisin at room temperature. ○: Without nisin; ●: with nisin

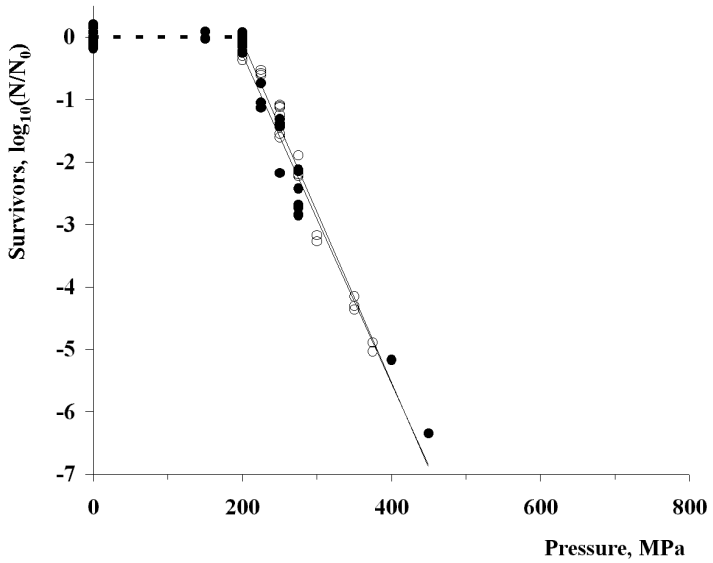

Fig. 3. Survivors of *Listeria monocytogenes* in minced beef as a function of high hydrostatic pressure and nisin at chilled condition (+ 4 °C)

○: without nisin; $\log_{10}(N/N_0) = 5.36–0.0272 \cdot p$; $n = 38$; $r^2 = 0.983$
●: with nisin; $\log_{10}(N/N_0) = 4.96–0.0262 \cdot p$; $n = 32$; $r^2 = 0.943$

2.3. The effect of treatments on B. cereus

The suspension of dormant spores of *B. cereus* was heat treated at 60 °C for 10 min (killing vegetative cells) and mixed with minced beef, and then nisin was added. The colony count of non-heat activated sample was reduced only by 1 log cycle at 800 MPa, which indicates a very high pressure resistance. The slope of the regression equation was –0.0013 ($\log_{10}$ CFU MPa^{-1}). Nisin did not improve the killing effect of pressure.
Two weeks after the treatment, the reduction of the cell count was close to 3 log cycles at 800 MPa compared to the initial cell count, and the slope of the regression equation was –0.0034 ($\log_{10}$ CFU MPa^{-1}) (Fig. 4).

As the spore suspension was heat activated (80 °C 10 min) and put into the minced beef, the colony count dropped by more than 1 log cycle at 800 MPa and was not influenced by the presence of nisin. Reduction of viable cell count was only 0.5 log cycle after 16 days at 4 °C. This difference between the colony counts before and after storage might indicate reparation of damaged spores (Fig. 5). The survival rate of treated spores after storage was higher in case of heat activated spores, than in case of non-heat activated ones (Figs 4 and 5). Efficiency of HHP combined with nisin needs to be further explored.

Since bacterial spores are generally very resistant to preservation methods, pressures higher than those applied in this experiment are necessary to reduce spore contamination sufficiently. Hence, the combined treatment has a high importance in the food preservation. Nisin is active against bacterial spores, inhibits their outgrowth, resulting in a stage which is almost as sensitive as the vegetative cells. The sub-lethal injury of spores was probably repaired during storage time, and this process was not influenced by nisin. According to ROBERTS and HOOVER (1996), high hydrostatic pressure treatment of spores of *Bacillus coagulans* (in other combination, higher temperature and longer pressurisation) was very effective. Further investigation is needed to study the spore survival.

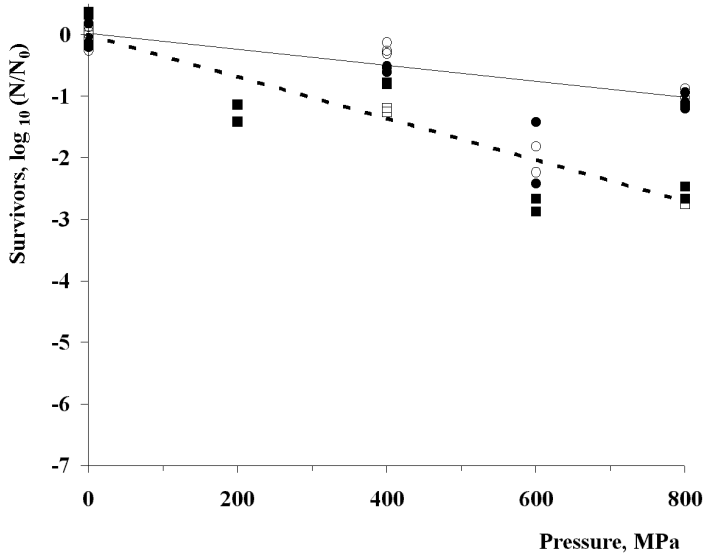

Fig. 4. Survivors of non-heat activated *Bacillus cereus* spores in minced beef as a function of high hydrostatic pressure (p) and nisin after 14 days storage at 4 °C

○: just after the treatment without nisin; $\log_{10}(N/N_0) = -0.0013 \cdot p$; $n = 21$; $r^2 = 0.868$
●: just after the treatment with nisin; (with and without nisin)
□: after storage without nisin; $\log_{10}(N/N_0) = -0.0034 \cdot p$; $n = 18$; $r^2 = 0.876$
■: after storage with nisin; (with and without nisin)

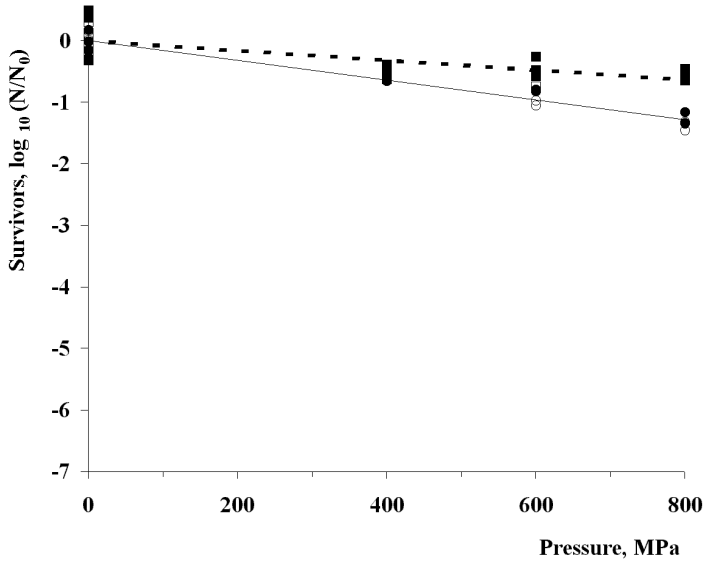

Fig. 5. Survivors of heat activated *Bacillus cereus* spores in minced beef as a function of high hydrostatic pressure (p) and nisin after 16 days of storage at 4 °C

○: just after the treatment without nisin;	$\log_{10}(N/N_0) = -0.0016 \cdot p$; $n = 20$; $r^2 = 0.943$
●: just after the treatment with nisin;	(with and without nisin)
□: after storage without nisin;	$\log_{10}(N/N_0) = -0.0008 \cdot p$; $n = 35$; $r^2 = 0.598$
■: after storage with nisin;	(with and without nisin)

3. Conclusions

The total viable cell counts in minced chicken meat were reduced with high efficiency in the range of 0–300 MPa hydrostatic pressure, and the presence of nisin further increased the killing effect. The sensitivity of pseudomonads is high to hydrostatic pressure, but nisin has no further effect on the survival.

The viable cell count of *L. monocytogenes* has scarcely changed as a function of hydrostatic pressure up to 250 MPa at room temperature and 200 MPa at chilled condition in minced beef. The presence of nisin only slightly influenced the result at room temperature. Over the threshold pressure level, the surviving fraction dropped sharply in case of chilled condition (D=37–38 MPa), regardless of the presence of nisin.

The dormant spores of *B. cereus* were very resistant in the pressure range investigated. Nisin had no additional effect. Heat activated spores behaved the same way, but after 16 days treated spores showed higher survival.

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