

*Original Research Paper***EFFECT OF HIGH PRESSURE TREATMENT ON *LISTERIA INNOCUA* IN DRY CURED FERMENTED MEAT PRODUCTS**

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The aim of this experimental work was to study the effect of pressure and time in fermented meat products of Czech origin inoculated with *Listeria innocua*. Physico-chemical characteristics (pH and a_w) were measured before pressure treatment. Dry-cured fermented ham - pršut neck and striploin were inoculated with *Listeria innocua* (strain 4030 CCM) at a level of approximately 10^6 cfu.g⁻¹. Vacuum packaged samples were treated by combination of pressure (400, 450, 500, 600 MPa) and time (1.5; 3; 6; 10 and 20 min). Organoleptic evaluation was performed before and after high pressure treatment. High pressure had a bactericidal effect on *L. innocua*: reduction higher than 5 log units was observed at a pressure of 600 MPa and time 20 min without organoleptic changes. These results have shown that high pressure treatment can enhance the microbiological quality of dry cured fermented ham.

Keywords: *Listeria*; high pressure treatment; fermented meat product; organoleptic evaluation; pršut.

INTRODUCTION

Problems with *Listeria monocytogenes* in foods have become more serious in recent years. Due to high mortality of infected persons, foodborne diseases caused by *Listeria* present a public health risk. Especially dangerous pathogen in food industry is *L. monocytogenes*. Its origin may be infected animals or poor sanitation practices. The disease in piglets and young pigs may result in nearly 100% mortality. It may hardly occur as bacteremia in case of transport disease, metabolic or immunological disorders. It used to be a result of secondary contamination (intestines etc.). *Listeriosis* is a notifiable disease, and is under strict control of the veterinary service. Pigs suffering from listeriosis must not occur as a source of meat contamination in meat industry directly.

The presence of *Listeria* in food is regarded as a major problem because it can cause serious illnesses and death (Jay, 1996; Ryser and Marth, 1999). The ability of *L. monocytogenes* to reproduce at refrigeration temperatures, survive a wide range of pH values and its incidence in foods affirm the importance of the inactivation of *L. monocytogenes* during food processing. Great care must be taken to ensure that any novel HPP (high pressure processing) treatments are capable of eliminating pathogens such as *L. monocytogenes* from foods. One of possible ways in case of fermented meat contaminated by *Listeria* is the use of HPP treatment. The use of high pressure for food preservation in comparison with traditional methods observe much more carefully

nutritional value, texture and especially palatability. At present, meat preservation by high pressure treatment has been used in Spain, USA, Japan, Italy and Germany. In the Czech Republic it has been used for preservation of fruit juices.

Ready-to-eat (RTE) dry cured fermented meat products, such as sliced dry-cured ham, are convenient and highly appreciated by the consumers. Their safety is ensured by decrease of water activity to below the growth limit of most pathogens, and low pH, enabling a higher efficacy of bacterial control in a 'hurdle technology' concept. Whereas lower water activity (a_w) is reached by the combined effects of salt uptake and meat shrinkage during ripening, pH decrease results from the primary process of microbial fermentation (Pipek, 1998). Pršut of Czech origin is a dry-cured meat product which is fermented, not thermally processed, matured at a constant temperature, relative humidity and exposed to cold smoke.

However, the manufacture of this type of food products increases the risk of microbial contamination, mainly during slicing and packaging. Among pathogenic microorganisms, *Listeria monocytogenes* constitutes the major concern of RTE products, since it is a widespread environmental microorganism and it is difficult to eradicate from the product environment. Additionally, *L. monocytogenes* is able to grow or survive in many foods during refrigerated storage. EPIDAT data show that in the Czech Republic, 25 cases of listeriosis in 2009 and 32 cases in 2010 were reported (SZÚ, 2011). According to

data from EFSA, the highest incidence of listeriosis in 2009 were 388 cases in Germany, 328 in France and 235 in United Kingdom (EFSA, 2011).

Consumers in the 21st century demand high quality foods that are free from additives, fresh tasting, microbiologically safe and with an extended shelf-life. One food technology that has the potential to meet these demands is high pressure processing. Moreover, there is an increasing worldwide interest in the use of HPP.

The major benefit of pressure represents its homogeneity of treatment at every point in the product since the applied pressure is instantaneously and uniformly distributed within the HPP chamber throughout different media, avoiding complications such as non-stationary conditions typical of convection-type and conduction-type processes (Ritz et. al. 2000a; Master et al., 2004).

High hydrostatic pressure processing is therefore the attractive new preservation technology that has the potential to meet the consumer demands in relation to high quality foods. Being mild for several types of food, HPP has the potential to inactivate pathogenic and spoilage microorganisms. This non-thermal technology shows a promising potential as listericidal treatment for RTE products and it is recognised by the Codex Alimentarius (CAC, 2007) and the FDA, Health and Human Services (HHS, 2008). HPP can be applied as a final preservation measure, after slicing and packaging, as an in-package “cold” pasteurization step, offering an additional microbiological safety level to RTE products.

The effectiveness of the HPP is known to depend on technological parameters, such as pressure, holding time and temperature, as well as the type and the physiological state of microorganisms (Hugas et al., 2002). Increasing pressure and treatment holding time result in an increase of microbial inactivation. However, the effectiveness of the treatment or the resistance of the microorganisms is extremely variable and it depends on (1) the process parameters (achieved pressure, treatment temperature and exposure time); (2) the strain (gram-positive microorganisms are more resistant to HPP than gram-negative species as well as spores), cell morphology (bacilli are more sensitive to pressure than cocci) and the stage of growth of the microorganisms (bacteria from the early log phase of growth are more baro-sensitive than cells from stationary, dormant or death phase); and (3) the meat matrix to be treated (Hugas et al., 2002; Saccani et al., 2004).

The object of this study was to assess the effect of high hydrostatic pressure on *Listeria innocua* in dry-cured fermented meat product, choose the best combination of high hydrostatic pressure and time, and to assess whether sensorial changes after pressurization occur. The gram-positive *L. innocua* was selected for this study. *Listeria*

monocytogenes and *Listeria innocua* are of very similar nature therefore for experimental purposes obviously a non-pathogenic strain is used.

There are many methods of listeria inactivation; in this report a specific method suitable for fermented meat products was studied. Therefore, in our experiments a meat producing enterprise also participated.

MATERIALS AND METHODS

Pršut neck and pršut striploin were obtained from retail. Samples were transported to the laboratory in cool boxes and then stored in laboratory refrigerator at a temperature of 0-4 °C.

Preparation of inoculum and inoculation procedure

Stock of *L. innocua* (strain CCM 4030) originating from the Czech Microbes Collection Institute in Brno was used for inoculation. Examination was performed in accordance with ČSN EN ISO 11290 – 1 (diagnosis of *Listeria*) and ČSN EN ISO 11290 – 2 (for count of *L. innocua*).

For revival, a freeze-dried culture was inoculated into liquid medium Fraser ½ and incubated at 37 °C for 48 hours. For concentration detection of *L. innocua* in inoculum revived cells were streaked onto solid diagnostic medium Oxford and AL – agar (BioRad, France).

Sample preparation

Sample homogenisation was done by the use of homogenizer KRUP. Before inoculation samples were tested for absence of *Listeria* according to ČSN EN ISO 11290 - 1. For experiments, the samples were inoculated at a final *L. innocua*, concentration at approximately 10^6 cfu.g⁻¹. Twenty-five grams of tested samples were placed into microtine bags, inoculated with *L. innocua* (collection strain CCM 4030) and homogenized in homogenizer STOMACHER (UK) and vacuum-packed. For organoleptic evaluation one part of samples was left without inoculation. ČSN EN ISO 11290 - 2 was applied for detection of inoculated *L. innocua* in the sample before and after high pressure treatment.

High pressure treatment

High pressure processing was performed on the day of inoculation. Vacuum-packed *Listeria innocua*-contaminated samples were subjected to different pressurization treatment. Pressurization was carried out combining different values of pressure (400, 450,

500, 600 MPa) and time (1,5; 3; 6; 10; 20 min). Each combination of treatments was performed ten times under the same conditions.

Enumeration of surviving cells

The number of cell was determined before and after treatment and the efficiency of treatment (bacterial inactivation) was assessed in terms of logarithmic reductions as the difference between counts after the treatments end the initial inoculum, i.e. $\log(N/N_0)$.

Physico-chemical analysis

pH and water activity were measured before HPP. Measurement of pH was performed by accredited method with the use of pH meter WTW 90 (Germany). Water activity (a_w) by accredited method with the use of Thermoconstanter TH-2 (Switzerland) was performed.

Organoleptic evaluation

Organoleptic evaluation was carried out by the expert panel. Following parameters were evaluated: appearance, consistency, colour, smell, taste, severability of slices. The samples were organoleptically assessed before and after the pressure treatment. For this part of the experiment the samples were not minced, hence there were evaluated the slices of ham, which were not contaminated with *L. innocua*.

Statistical analysis

Data were statistically analysed using one-way analysis of variance (ANOVA), and means were separated by Tukey-test significant difference test at 5% significance level. Data analyses were conducted using the statistical package STATISTICA 7.0.

RESULTS AND DISCUSSION

Physico-chemical analysis

Obtained average of pH pršut neck and pršut striploin were from 5.63 to 5.98 and 5.51 – 5.89, respectively. The pH of pršut striploin was lower than that of pršut neck. In the past, the effect of pH was demonstrated (Alpas et al., 2000; Mackey et al., 1995; Stewart et al., 1997). Low food pH is a major advantage in high-pressure inactivation procedures (Hoover et al., 1989; Roberts and Hoover, 1996).

According to Mackey et al. (1995) the mean advantage

in efficiency of high pressure treatment of *Listeria monocytogenes* was at pH 5.6 – 7.00. The results of pH varied within this scope in our experiment.

Water activity was from 0.87 to 0.91 (pršut neck) and from 0.79 to 0.90 (pršut striploin). Petran and Zottola (1989) reported that the optimum a_w for the growth of *Listeria monocytogenes* is ≥ 0.97 . Pathogen growth limit is at 0.92 (Hereau, 2011). The obtained values show that these products do not support significant growth of *L. monocytogenes*. A low pH value and a high a_w value make vegetative microbial cells more sensitive to a high-pressure treatment (Carlez et al., 1993; Arroyo et al., 1997).

High pressure treatment

One of the most important observations in this experiment was the statistically significant ($P < 0.05$) relationship between inactivation of *L. innocua* population and the two variables of high pressure treatment (pressure and time) at 95% confidence level. The effect of pressure on bacterial inactivation has been well documented (Hoover et al. 1989, Simpson and Gilmour 1997). It was determined that inactivation of *L. monocytogenes* is dependent upon the pressure level. Our study confirms the positive effect of pressure.

Effect of pressure

Fig. 1 (a – pršut neck, b – pršut striploin) shows the relation between the increase in pressure and reduction of bacterial population. The curves expressing this relation were almost sigmoidal. There was a statistically significant difference ($P < 0.05$) between the low (400 MPa) and high (600 MPa) pressure. The treatment efficiency improved as the pressure increased. The same observation on the efficacy of pressure reported Ritz et al. (2000 a).

Effect of treatment time

The treatment of time was also found to be significant in bacterial inactivation. Time rise corresponds to greater inactivation of the *L. innocua* (Fig 2a – pršut neck, 2b – pršut strioloin). Yuste et al. (1999) stated that the time seemed to be an important variable value for microbial inactivation under some conditions.

Effect of interaction of treatment pressure and time

Figure 3a – pršut neck, 3b – pršut striploin presents the curves of pressure levels at different times. Bacterial inactivation was the greatest at 600 MPa, 20 min.

Figure 1a: Effect of pressure on *L. innocua* (pršut neck)

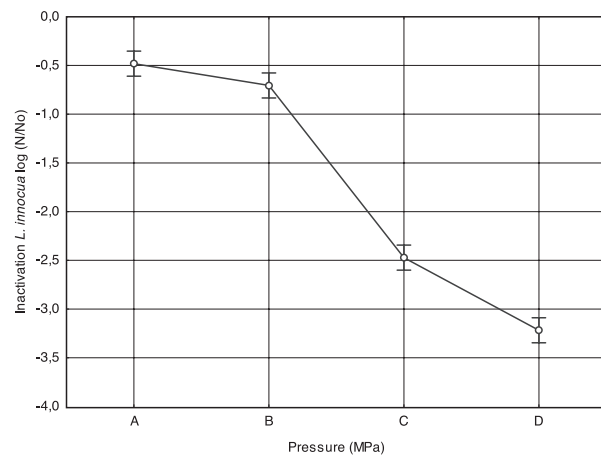


Fig. 2a: Effect of time on *L. innocua* (pršut neck)

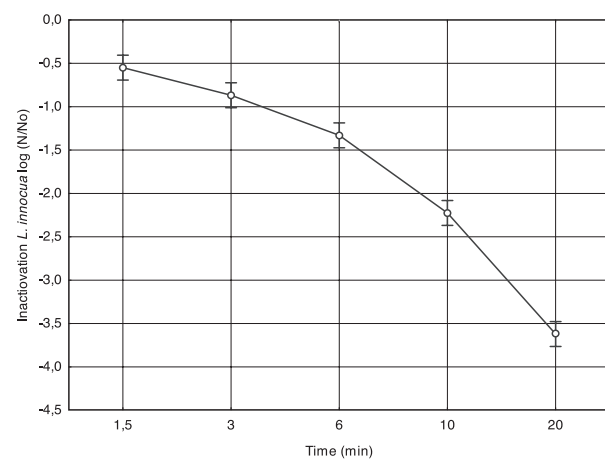


Figure 1b: Effect of pressure on *L. innocua* (pršut striploin)

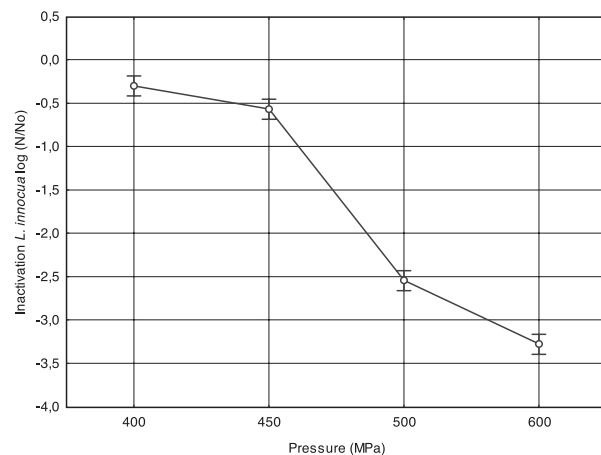
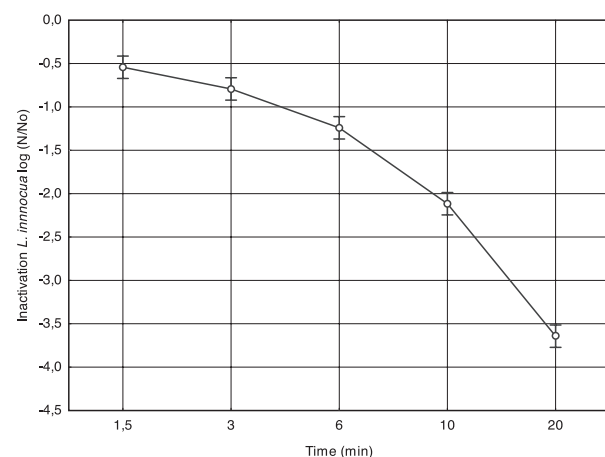


Fig. 2b: Effect of time on *L. innocua* (pršut striploin)



Treatment of pršut neck and pršut striploin at 500 MPa and 600 MPa for 20 min caused more than 5 log and 6 log *Listeria* reduction, respectively. These results comply with European Regulation (CE) 2073/2005 amended by Regulation (CE) 1441/2007.

The difference in efficiency between the treatments 500 a 600 MPa 20 min was not significant. Treatment at 600 MPa for 10 min caused reduction more than 4 log *Listeria innocua* in both samples of pršut. Ritz et al. (2000 b) observed a complete inactivation of *Listeria innocua* under similar conditions of treatment (600 MPa, 10 min).

Pressure at 600 MPa for 10 min caused a significant reduction of *L. innocua* (more than 4 log) in both samples of pršut (neck and striploin), but did not cause complete inactivation. Ritz et al. (2000b) investigated the virulence of *Listeria monocytogenes* before and after treatment with high pressure. It was observed that the pressure 600 MPa/10 min for 20 °C caused a complete inactivation of *L. monocytogenes*.

The results of this experiment showed that the inactivation of *L. innocua* ensuring wholesomeness of the food product, a pressure from 500 to 600 MPa for 20 min exposure is needed. In general, low water activity protects cells against pressure. Microorganisms injured by pressure are more sensitive to low water activity (Cheftel and Culioli, 1997). Lower water activity allows for the bacteria to become resistant to high pressure (Rubio, 2007), therefore low water activity in the examined samples may have caused the need for higher pressure and time compared to previous research conducted by Garriga et al. (2004).

Organoleptic evaluation

The following parameters were assessed: appearance, consistency, colour, smell, taste, severability of meat slices. No visual differences were observed between NT and HPP samples. These results have shown that HPP did not have a negative effect on sensorial quality of pršut.

Figure 3a: Effect of interaction pressure and time (pršut neck)

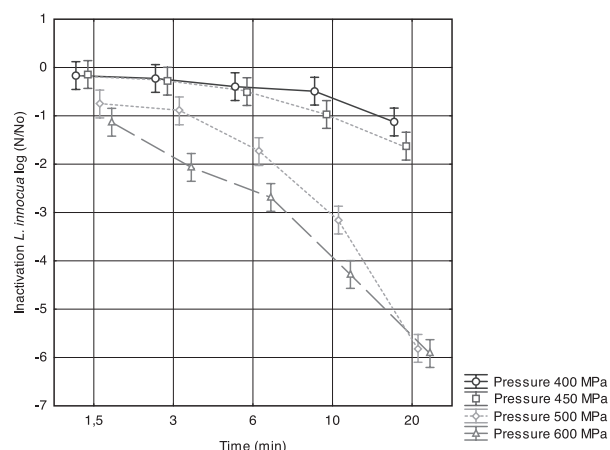
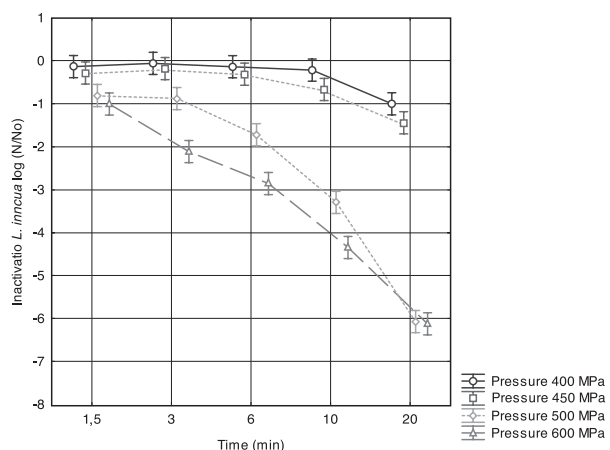


Fig. 3b: Effect of interaction pressure and time (pršut striploin)



According to Karlowski (2002), high pressure causes an increase in lightness and reduction in redness of smoked pork loin but on the other hand, in the two types of ham samples, pressurized at up to 500 MPa it has been found that the colour was visually identical to that of non pressurized ham.

CONCLUSIONS

High pressure processing at 500 MPa and 600 MPa for 20 min was an efficient method for delaying the growth of *Listeria innocua* in pršut.

Based on these data, HPP would be an effective alternative to heating, freezing or curing for control of *Listeria* in pršut. Moreover, when applied after fermentation and drying, HPP was also effective at reducing numbers of *Listeria* organisms. These findings are important in the context of HPP being used as a final treatment to prevent lethality of *Listeria* in foods.

Although the initial capital expenditure is still high, pressure treatments require less energy than thermal processing and results in no sensorial changes of fermented meat products. Therefore such products would be commercially competitive.

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