HIGH PRESSURE INACTIVATION OF *LISTERIA MONOCYTOGENES* IN DRY CURED MEAT PRODUCTS

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Abstract

Diseases contracted by Listeria monocytogenes, listeriosis, a serious infection caused by eating food contaminated with the bacterium Listeria monocytogenes, have recently been recognized as an important public health problem in the Czech Republic. Cured meat product can be recontaminated by exposure after the lethality treatment during the peeling, slicing, repacking and other procedures. If the Listeria is present on the equipment used for peeling, slicing or repacking, then pathogens can be transferred to the product upon contact for that reason it is necessary to follow the microbiological quality of dry cured meat products) immediately after the treatment and then in regular time periods during the storage process. As heat treatment is not suitable for this product, an alternative process to minimise the microbiological counts is necessary. High pressure treatment is a new preservation method without high temperatures, avoiding desirable alternation used by thermal treatment of food such as vitamin loss, reduced bioavailability of essential amino acids, flavour loss, modification of taste and colour.

Key words: *Listeria monocytogenes*, high pressure, listeriosis, dry cured meat products

INTRODUCTION

Listeria is widely recognized as an opportunistic food-borne pathogen. Consumption of foods contaminated with *Listeria monocytogenes*, poses significant health risks to humans (Siegman-Igra et al., 2002). Listeriosis is an important food-borne zoonosis due to the severity of the disease and high mortality. It is a significant risk factor in well-defined groups: immunocompromised individuals, pregnant women and neonates younger than four weeks (Koch, Stark, 2006). Listeriosis is a relatively rare infection in humans but the severity and mortality rate can reach up to 50 % (Low, Donachie, 1997).

For more than 30 years up to 2006, the incidence of human listeriosis in the Czech Republic has been low, ranging between 0.1 and 0.2 cases per 100 000 population per year. In 2006, however, the incidence of human listeriosis was about 0.7. Altogether, 75 cases of listeriosis were reported to have occurred in 2006 (Vít et al., 2007). The number of cases of listeriosis in EU increased to 1,583 in 2006 compared to 1,427 in 2005. Furthermore, the incidence rates of this infection in Europe have shown a statistically significant increase over the past five years (Denny, McLauchlin, 2008).

Meat and meat products have frequently been contaminated with *L. monocytogenes* and may serve as vehicle of other pathogenic organisms.

MICROBIOLOGY OF GENUS *LISTERIA*

Listeria spp. are gram-positive rods 0.4 to 0.5 μm in diameter and 0.5 to 2 μm in length. Members of the genus *Listeria* are aerobic and facultatively anaerobic, do not produce spores, and demonstrate characteristic motility when cultured at 20 to 25°C. Colonies are bluish gray by normal illumination, but a blue-green sheen is visible by oblique light. *Listeria spp.* can grow between pH 6 and pH 9 and in temperatures ranging from 1 to 45°C. Optimum growth occurs between 30 and 37°C. *Listeria spp.* are catalase positive, oxidase negative, methyl red positive, and Voges-Proskauer positive (Farber, Peterkin, 1991).

The genus *Listeria* includes six species, and these comprise *L. monocytogenes*, *L. innocua*, *L. welshimeri*, *L. seeligeri*, *L. ivanovii* and *L. grayi*. Listeriosis is a disease caused by bacteria of the genus *Listeria*, and *L. onocytogenes* is the major pathogenic species in both animals and human (McLauchlin, Jones, 1999).

CHARACTERISTIC OF *LISTERIA MONOCYTOGENES* IMPORTANT TO FOOD PROCESSOR

The control of microorganisms is one of the most important aspects of food preservation. Bacterial destruction ensures food safety, but it also involves the application of more intense treatments that may cause additional food quality losses (Ray, 1986).

Listeria is a pathogen, with the ability to adapt to a wide range of conditions such as refrigeration temperatures (2–4°C), acidic foods, foods with high salt content (Rocourt, Cossart, 1997).

Survival at low temperatures

L. monocytogenes has the ability to grow over a wide range of temperatures (2–45°C). Its survival and growth

at refrigeration temperatures (2–4°C) are two of the many factors that make the control of this foodborne pathogen difficult (Rocourt, Cossart, 1997). Since refrigeration is one of the most common ways to increase the shelf life of foods, understanding the mechanisms behind its survival and growth at low temperature could provide information to help develop more effective control methods for the pathogen.

Survival at low pH

Like a many other bacteria, *L. monocytogenes* grows optimally at a pH close to neutrality. Growth of L. monocytogenes has been reported at pH values ranging from 4.0 to 9.6 (Phan-Thanh, 1998). In the absence of other growth limiting factors, highest final populations were reached at pH 6.0 to 8.0 (Buchanan et al., 1994). Absence of growth and decrease of in cell viability may be observed at pH ≤ 5.5, when the other environmental conditions (e.g. temperature) are not optimal for survival of *L. monocytogenes*. Low pH je one of the most critical safety and quality determinants of fermented foods.

Survival at low water activity

Like most bacteria, *L. monocytogenes* grows optimally at aw ≥ 0.97 (Petran, Zottola, 1989). Drying and addition of salt or sugar are traditional methods to lower water activity of food and therefore enhance its shelf life. *Listeria monocytogenes* survives or even grows in food with relatively low water activity (Nolan et al., 1992).

Survival at extreme salt concentration

Listeria monocytogenes tolerates extremely high salt concentration. The ability of *Listeria* to adapt and survive in high concentrations of salt makes it difficult to control the pathogen in foods. *L. monocytogenes* can grow in the presence of salt concentrations of 10% (w/v) in a rich medium such as brain heart infusion (BHI) broth, which contains osmoprotectants such as betaine, carnitine, and peptides (Beumer et al., 1994).

Resistance to bacteriocins

Antimicrobial peptides produced by bacteria such as lactic acid bacteria are called bacteriocins. They are synthesized from ribosomes and are effective against closely related bacteria (Klaenhammer, 1993). Nisin is a bacteriocin produced by *Lactococcus lactis* strains and is approved for use in food preservation in many countries; however, several other bacteriocins have shown the potential for future applications in food systems (Cleveland et al., 2001). Since its discovery and approval for use in foods, nisin has been used as a preservative in the dairy and meat industries to control pathogens such as *L. monocytogenes*.

INCIDENCE OF LISTERIA MONOCYTOGENES IN MEAT PRODUCTS

L. monocytogenes can be found in a wide variety of raw and processed meat and meat products such as beef, pork, fermented sausages, fresh produce such as radishes, cabbage, seafood and fish products have all been associated with *Listeria* contamination (Rocourt, Cossart, 1997). Meat is a very rich medium for growth of microorganisms, it is mainly constituted by water, protein (15–21%), fat (0.5–25%), oligonutrients and vitamins (especially rich in B group vitamins) (Cheftel, 1995).

The incidence of *Listeria* in fresh meat may vary from 0 to 68%, while in processed meat products, including ready-to-eat food, the contamination ranges from 8 to 92% (Johnson et al., 1990). However, a higher incidence of *L. innocua* in meat products, compared to *L. monocytogenes* was reported (Pini, Gilbert, 1988).

In the Community Summary Reports on Trends and Sources of Zoonoses in the EU in 2004, 2005 and 2006 the overall ranges of contamination of foods with *L. monocytogenes* were (presence in 25 g): 0-48% in meat products, 0–40% in poultry meat product, 0–30% in fish products (EFSA, 2007).

Fermented meat products

Fermented meat products have moderate rates of consumption in many countries. These products contain lactic acid, salt and nitrite that prevent the growth of *L. monocytogenes* and, in fact, cause inactivation of the pathogen during storage, particularly storage at room temperature. Because of the contamination of the raw meat ingredients, these products have moderate contamination rates at retail. Storage times can be very lengthy. Fermented meat products are often contaminated with *Listeria* and are produced without any lethal processing step, but their final composition prevents growth of the microbe during storage (WHO, 2004).

In dry cured Canadian sausages, 10 out of 42 samples were positive for *Listeria monocytogenes* before fermentation and five of them remained positive even after the maturation period (Farber et al., 1988).

The presence of a typical microflora in combination with physico-chemical properties of cured meat should prevent Listeria growth in cured meat products. Therefore, the health risk associated with the occurrence of Listeria in cured meat products can be regarded as low. Nevertheless, food laws require that the bacterium be absent in ready-to-eat products. To the end, makers must ensure that their products are not contaminated by pathogens. They have to control the fermentation process, and make appropriate use of the drying processes in order to reduce any pathogens potentially present (Silvana, Giovanni, 2002).

MICROBIAL INACTIVATION BY NEW TECHNOLOGY OF FOOD PRESERVATION – HIGH HYDROSTATIC PRESSURE

Listeria is killed by thermal treatment, but heat treatment is not suitable for dry cured meat products. Regarding these aspects it is necessary to implement new preservation technique, which will be effective and a mild method to the inactivation of *Listeria* in sliced cured meat products. In this sense, high hydrostatic pressure processing is a very promising preservation technology of sliced meat cured product, which can be applied to the product after slicing and vacuum packaging. HHP at low or moderate temperature causes destruction of microbial vegetative cells without remarkable changes in odour, taste and nutrient content (Hugas et al., 2002).

Furthermore, the use of HHP will ensure the safety of meat cured products and extends the life of product. However, the effectiveness of the treatment or the resistance of the microorganisms is extremely variable and it depends on the process parameters (achieved pressure, treatment temperature and exposure time); the strain (gram-positive microorganisms are more resistant to HHP 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 barosensitive than cells from stationary, dormant or death phase); and the meat matrix to be treated (Hoover et al., 1989). Cells of bacteria subjected to stress other than pressure (e.g. sublethal heat, cold-shock) become more resistant to pressure. Stress is induced during the stationary phase of growth through starvation or acidification (Archer, 1996).

High pressure processing uses an isostatic pressure at room temperature and between 100 and 600 MPa. In general, HHP at low or moderate temperature causes destruction of microbial vegetative cells and enzyme inactivation, without changing the organoleptic characteristics of the product and leaving the vitamins intact. The HHP treatments can induce special effects on the texture of product and structure of a given food and accordingly can be used for the development of new products or to increase the functionality of some ingredients (Hugas et al., 2002).

Among the pathogenic non-sporeforming gram-positive bacteria, *Listeria monocytogenes* one of the most well studied regarding the use of HHP processing. High-pressure inactivation of *Listeria* follows a second-order relationship with processing temperature. Food with high levels of proteins or glucose favored survival of the pathogen during the pressure treatment. The level of fat in food did not consistently affect survival of Listeria (Simpson, Gilmour, 1997). Food formulation containing bacteriocins (Kalchayanand et al., 1998), the lactoperoxidase system (Garcia-Graellset et al., 2000) carvacrol (Karatzas et al., 2001) synergistically enhance the action of high pressure processing against *Listeria*. Sensitivity to high pressure varied among *Listeria* strains (Garcia-Graellset et al., 2000, Tay et al., 2003).

Pressure ≥ 200 MPa causes irreversible protein denaturation, rupture of cytoplasmic membrane and leakage of cell contans (Lado, Yousef, 2002). This range of pressure also induces autolysis of *Listeria*. Expression of cold-shock protein is induced in survivors of high pressure processing (Masschlack et al., 2002). During storage, expression of cold-shock proteins also enhances the cell's resistance to high pressure (Wemekamp-Kamphuis et al., 2002). *Listeria* appear to be more sensitive to the effects of pressurization at low temperature (Carlez et al., 1993, Ritz et al., 2000).

There are a few published reports dealing with the inactivation of *Listeria monocytogenes* by high pressure processing, especially the inactivation of *Listeria monocytogenes* in cured ham.

Smelt (1998) supposes that inactivation pathogen microorganisms is result of combination of high pressure processing, time and temperature. Rubio et al. (2007), Garriga et al. (2004) and Hugas et al. (2002) envisaged effect of high pressure preservation in cured ham. Each of them used the same high pressure values 500–600 MPa, but at different times and temperatures during the high pressure treatment. This is also recognised by Hoover et al. (1989), Hugas et al. (2002), Saccani et al. (2004), the effectiveness of the treatment or resistance of the microorganisms depends on the process parameters (achieved pressure, treatment temperature and exposure time and the strain, cell morphology and the stage of growth).

There are no published reports available on toxicity studies of HHP-treated foods. It is well known that HHP can modify the activity of some enzymes and the structure of some proteins. Although covalent bonds are not affected, hydrogen bonds as well as hydrophobic and intermolecular interactions may be modified or destroyed (Hugas et al., 2002).

CONCLUSION

On the grounds of the above facts experiments aiming to find out relation between the use of high pressure under defined temperature and time on survival of *Listeria monocytogenes* were performed. For experiments cured meat products of Czech origin were used. At the present time methods used and results are subject of evaluation and are to be presented in the next article.

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