

Influence of Modified Atmosphere Packaging on the Survival of *Salmonella* Enteritidis PT 8 on the Surface of Chilled Chicken Legs

Radka Hulánková, Gabriela Bořilová, Iva Steinhäuserová

Department of Meat Hygiene and Technology, Faculty of Veterinary Hygiene and Ecology, University of Veterinary and Pharmaceutical Sciences Brno, Czech Republic

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Abstract

The aim of this study was to find whether low numbers of *Salmonella* in the presence of natural microflora will survive on the surface of chicken legs stored in 30% CO₂/70% N₂ and 20% CO₂/80% O₂. In four experiments, a total of 240 pieces of chicken leg were inoculated with a *Salmonella* Enteritidis, PT 8, wildtype strain resulting in initial concentrations of approximately 4 log, 2.5 log, 1.5 log and 0.5 log cells per piece and kept under selected modified atmospheres for 14 days. Counts of *Salmonella* were determined by the Most Probable Number method (MPN) at days 0, 3, 7, 10 and 14 of storage. No significant increase or decrease in *Salmonella* numbers was observed in the atmosphere of 20% CO₂/80% O₂. In the atmosphere of 30% CO₂/70% N₂ there was a significant decrease in cell numbers at days 10 and 14; however, this decrease was proved only in experiments with an initial *Salmonella* concentration of 4 log and 1.5 log cells per piece. We proved that even low numbers of *S. Enteritidis* in the presence of natural microflora survive well on the surface of poultry stored at 3 °C in both modified atmospheres we tested. In the case of temperature abuse even products with low initial numbers of *Salmonella* may constitute a health risk for consumers.

Enumeration, Enteritidis, chicken legs, MPN, MAP

Poultry meat is very popular among consumers, though it is also the type of meat with the highest risk of the presence of *Salmonella*. In addition to eggs and egg products, poultry meat and poultry meat products are considered one of the main sources of salmonellosis, the incidence of which in the Czech Republic is continually one of the highest in the European Union. The most commonly identified causative agent of salmonellosis, as well as the most commonly isolated *Salmonella* serotype in poultry meat, is *Salmonella* Enteritidis (EFSA, 2009).

Salmonella contamination of poultry meat at the slaughterhouse can be caused by faeces released from the alimentary tract of birds colonised with *Salmonella*, followed by cross-contamination resulting in the spread of bacteria to previously uncontaminated carcasses during processing. The processing operations including final spray-washing and chilling cannot completely remove the *Salmonella* from the surface of the carcasses (Mead 2004; Olsen et al. 2003; Rasschaert et al. 2007). Some *Salmonella* strains can survive in the slaughter environment for up to 5 days despite daily cleaning and disinfection procedures (Olsen et al. 2003) and results of studies using genotyping methods indicate that not only the currently slaughtered birds but also the slaughter environment can be an important source of poultry meat contamination (Rasschaert et al. 2007).

The reported prevalences of *Salmonella* spp. on broiler carcasses after chilling range widely, reaching several tens of percents in some cases (FAO/WHO 2002) depending, for example, on the presence/absence of *Salmonella* within the slaughtered flock, the processing technologies used, the effectiveness of cleaning and washing at the slaughterhouse, and the microbiological methods used for *Salmonella* detection. Unlike another important pathogen *Campylobacter* spp., the numbers of *Salmonella* in poultry are generally not very high. Although *Salmonella* enumeration is not usually performed, in most of the studies

Address for correspondence:

Mgr. Radka Hulánková
Dept. of Meat Hygiene and Technology
University of Veterinary and Pharmaceutical Sciences
Palackého 1-3
612 42 Brno, Czech Republic

Phone: + 420 541 562 747
E-mail: h06294@vfu.cz
<http://www.vfu.cz/acta-vet/actavet.htm>

conducted so far the *Salmonella* counts have not exceeded 2 log MPN/carcass, rarely 4 log MPN/carcass (FAO/WHO 2002; Mead 2004).

One of the most important aspects in poultry meat production in addition to safety is shelf life. Both these indicators can be affected positively by modified atmosphere packaging (MAP) in combination with low storage temperature (< 4 °C) (Phillips 1996). Because of the quick turnover time in the retail marketing of poultry meat and the extra costs of MAP, modified atmosphere packaging has not been frequently employed recently; however, from an economical and public health point of view, interest among producers is increasing (Bolder 2007).

The most frequent gaseous mixtures used for poultry packaging are 100% CO₂ and 25-30% CO₂/70-75% N₂ or 20-40% CO₂/60-80% O₂ (Phillips 1996). All these mixtures take advantage of the antimicrobial effect of carbon dioxide manifested at concentrations above 10% (Floros and Matsos 2005). A CO₂ concentration of 20-40% is generally used, as concentrations under 15% do not inhibit microbial growth sufficiently, whereas concentrations above 40% can lead to the collapse of packages due to CO₂ absorption by meat tissue (McMillin 2008).

Carbon dioxide inhibits the growth of aerobic microorganisms by prolongation of the lag phase and generation time during the logarithmical phase of growth (Floros and Matsos 2005). Gram-negative bacteria are generally more sensitive to carbon dioxide than Gram-positive bacteria, which are mostly facultatively or strictly anaerobic (McMillin 2008). Increased levels of carbon dioxide also inhibit the growth of *Enterobacteriaceae*, including that of *Salmonella* (Floros and Matsos 2005; Phillips 1996).

It is well known that the amount of dissolved carbon dioxide and its antimicrobial effect increase as storage temperature decreases. Although *Salmonella* spp. should not be able to multiply at the temperatures under 4 °C used for poultry meat storage according to the requirements of the legislation, cases of *Salmonella* growth in poultry meat at 2 °C have been reported (D'Aoust 1991). Many predictive models for *Salmonella* growth in poultry meat have been developed. Nevertheless, none of them is applicable for temperatures under 4 °C (FAO/WHO 2002).

Even low numbers of *Salmonella* can constitute a risk for consumers when temperature abuse occurs in the commercial chain allowing the bacteria to multiply, and in the case of inadequate thermal processing. The aim of this study was, therefore, to find out whether even low numbers of *Salmonella* in the presence of natural microflora on the surface of chicken legs will survive storage in 30% CO₂/70% N₂ and 20% CO₂/80% O₂.

Materials and Methods

Chicken legs

A total of 240 pieces of chicken legs of the same weight category (average weight about 250 g) were obtained from a local poultry slaughterhouse. The chicken legs originating from a ROSS 308 broiler line were taken at the end of the slaughter line after chilling and transported at a temperature below +3 °C to the laboratory for immediate processing. For each combination of concentration and modified atmosphere a separate experiment with 30 chicken legs was performed.

Testing strain and preparation of inoculum

For inoculation we used a strain of *Salmonella enterica* subsp. *enterica* ser. Enteritidis, phagotype PT 8 (wildtype), isolated from poultry meat and provided by the National Institute of Public Health Brno. The inoculum was prepared from cells in the stationary phase of growth, obtained from a 24-h-old culture grown on blood agar at 37 °C (Oxoid, Great Britain; Bioveta, Czech Republic). Suspensions corresponding to the McFarland turbidity standard no. 1 and subsequent dilutions in saline were prepared to achieve the target inoculum concentration. In the first experiment, suspension of a concentration of about 4.5 log cells/ml was used, in the subsequent experiments the concentration was progressively decreased to 3 log, 2 log and 1 log cells/ml.

Inoculation, packaging, storage

Chicken legs were placed individually into sterile trays and their surface inoculated with bacterial suspension (0.5 ml/piece) spread by a sterile curved glass rod. Each tray was wrapped in AMILEN foil (Verpackungen

GmbH, Germany) (PA/PE 20 $\mu\text{m}/60 \mu\text{m}$, O_2 permeability $50 \text{ cm}^3\cdot\text{m}^{-2}$ at 24 h 23 °C 75% r.h., CO_2 permeability $150 \text{ cm}^3\cdot\text{m}^{-2}$ at 24 h 23 °C 0% r.h., water vapour permeability $3.0 \text{ g}\cdot\text{m}^{-2}$ at 24 h 23 °C 85% r.h.). The bags were flushed with commercial gas mixtures 30% $\text{CO}_2/70\% \text{ N}_2$ or 20% $\text{CO}_2/80\% \text{ O}_2$ (Linde Gas, Czech Republic). The samples in 30% $\text{CO}_2/70\% \text{ N}_2$ were packed with a VacStar S223 packing machine (Switzerland), the samples in 20% $\text{CO}_2/80\% \text{ O}_2$ were packed with a Turbovac 320-ST-S packing machine (Netherlands) and stored at $3.1 \pm 0.3 \text{ °C}$ for 3 to 14 days.

Microbiological examination

Samples were analysed on days 0 (after *Salmonella* inoculation and attachment), 3, 7, 10 and 14 of storage. Each chicken leg was transferred into a sterile polyethylene bag and rinsed in 100 ml of buffered peptone water (Oxoid, Great Britain). The enumeration of *Salmonella* in rinses was performed by the tree-tube most-probable-number (MPN) method described for *Salmonella* by Malorny et al. (2008). RVS broth and XLD agar were used (Oxoid, Great Britain) as the selective media. Numbers of *Salmonella* in 1 ml of rinse were calculated with a Microsoft Excel 2003 computer program using the Thomas formula (Blodgett 2008) and adjusted to MPN per piece.

Statistical analysis

The *Salmonella* counts were converted to log MPN per piece for the purpose of statistical analysis. Statistical analysis was performed by a StatPlus 2008 Professional v. 5.2.5.0 computer program. With regard to the nature of data we used a Kruskal-Wallis ANOVA non-parametric test for comparison of days within one concentration and atmosphere and for comparison of the atmospheres. A level of $p < 0.05$ was considered significant.

Results and Discussion

In addition to temperature and atmosphere composition, the survival and growth of *Salmonella* is influenced by several other factors, including pH and competitive microflora. Several authors have reported the inability of *Salmonella* Enteritidis to compete successfully with lactic acid bacteria (De Fernando et al. 1995; Nychas and Tassou 1996). When modelling the growth of *S. Typhimurium* DT104 on chicken meat with the presence of natural microflora, Oscar (2007) found that the initial concentration of *Salmonella* is an important factor in growth rate. The results of another study suggest that the generation time of various serotypes and even phagotypes of *S. Enteritidis* varies considerably at temperatures approaching the minimum growth temperature of *Salmonella* (Fehlhaber and Krüger 1998).

Salmonella survival on chicken legs is presented in Fig. 1 as log MPN *Salmonella* per piece (mean \pm SD). This figure shows that throughout storage there was no remarkable increase or decrease in *Salmonella* counts for any of the concentrations and atmospheres used.

In respect of the statistical analysis there were no significant differences found between days of storage in an atmosphere of 20% $\text{CO}_2/80\% \text{ O}_2$ for any of the initial concentrations. In the atmosphere of 30% $\text{CO}_2/70\% \text{ N}_2$ there were significant differences ($p = 0.043$) between days 7 and 10 of storage in experiment No. 1 (initial concentration 4 log MPN *Salmonella*/piece) and between days 3 and 10 ($p = 0.021$) and 3 and 14 ($p = 0.033$) in experiment No. 3 (initial concentration 1.5 log MPN *Salmonella*/piece). In experiment No. 4 (initial concentration 0.5 log MPN *Salmonella*/piece) values from day 3 and 14 were significantly different ($p = 0.047$). We found no significant differences between the modified atmospheres.

Several researchers have investigated the influence of this atmosphere on spoilage microorganisms in poultry meat. In general, modified atmospheres with an elevated CO_2 concentration slow the growth of typical aerobic microflora, which is replaced by more resistant microorganisms such as lactic acid bacteria and related species. However, members of the family *Enterobacteriaceae* also compose a substantial part of the microflora (Jiménez et al. 1997). Similarly Balamatsia et al. (2006) observed an increase in *Enterobacteriaceae* counts in raw poultry meat stored at 4 °C. Studies on the survival of *Salmonella* spp. in an atmosphere of 30% $\text{CO}_2/70\% \text{ N}_2$ are limited, but Sawaya et al. (1995) noted growth of *Enterobacteriaceae* on naturally contaminated chicken meat after 3 days of storage at 2 °C and in 30% $\text{CO}_2/70\% \text{ N}_2$ with numbers increasing by 3 log cycles

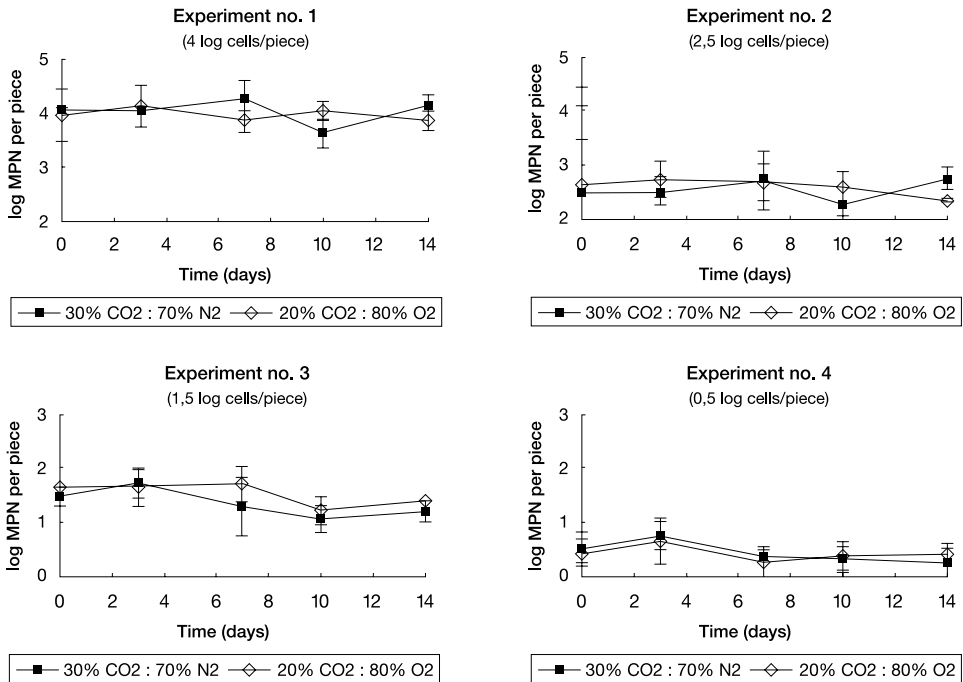


Fig. 1. Survival of *Salmonella* Enteritidis on chicken legs stored in modified atmospheres in relation to initial *Salmonella* concentration (mean \pm SD)

after 15 days. *Salmonella* spp. composed about 12% of *Enterobacteriaceae* members and the proportion remained constant throughout storage.

In the Czech Republic, mixtures of oxygen and carbon dioxide are widely used for whole poultry and poultry portion packaging. This modified atmosphere is also used by the poultry processing plant where the samples for this study were taken. Atmospheres composed of 25–90% O₂ and 15–80% CO₂ may be used, although 80% O₂/20% CO₂ is the most common gas mixture (McMillin 2008). High O₂ atmospheres were developed for red meat packaging because the presence of oxygen maintains the myoglobin in its oxygenated form, thus giving the meat the bright red colour expected by consumers (Phillips 1996). The presence of oxygen is not essential for poultry, and in the case of turkey meat may even lead to differences in taste and smell (Floros and Matsos 2005). High levels of oxygen inhibit the growth of anaerobic bacteria, though it is also necessary to limit the growth of aerobic Gram-negative spoilage microorganisms (such as pseudomonads) with carbon dioxide (D'Aoust 1991).

The influence of high O₂ atmospheres on *Salmonella* growth in chicken meat has been demonstrated by Nychas and Tassou (1996), who investigated the growth of *S. enteritidis* in artificially inoculated chicken breast stored at 3 °C in 20% CO₂/80% O₂. The initial concentration of 4.5 log CFU/g did not change markedly throughout a 12-day storage period.

Bacteriostasis of inoculated *Salmonella* cells by a combination of low storage temperature and carbon dioxide was also established in both modified atmospheres in our study. There was a significant decrease of *Salmonella* counts in an atmosphere of 30% CO₂/70% N₂ after 10 or 14 days of storage, respectively, but this decrease was observed only in experiments with an initial concentration of *Salmonella* cells of 4 log and 1.5 log cells per piece. This

could be caused for example by higher initial load of background microflora and its delayed development. What more, in spite of its importance for growth modelling and quantitative microbial risk assessments, *Salmonella* enumeration is not performed routinely. The MPN technique is particularly useful for enumeration of low concentrations of *Salmonella* cells. It is worthwhile noting that this method is extremely laborious (Malorny et al. 2008), so it is difficult to examine a large quantity of samples simultaneously, which negatively affects the power of the statistical tests.

Our results show that the initial concentration of *Salmonella* cells had no effect on their survival. We proved that low numbers of *S. Enteritidis* accompanied by natural microflora survive well on the surface of poultry stored at temperatures under 4 °C in both tested modified atmospheres, with a slightly better inhibition in the atmosphere with higher carbon dioxide concentration. In the case of more suitable growth conditions when a temperature abuse occurs in the commercial chain even products with low initial numbers of *Salmonella* cells can thus constitute a health risk for consumers.

Vliv modifikované atmosféry na přežívání *Salmonella* Enteritidis PT 8 na povrchu chlazených kuřecích stehen

Cílem naší práce bylo zjistit, zda i nízký počet salmonel v přítomnosti doprovodné mikroflóry přežije na kuřecích stehnech v průběhu skladování v atmosférách 30% CO₂/70% N₂ a 20% CO₂/80% O₂. Ve čtyřech pokusech bylo celkem 240 kusů kuřecích stehen naočkováno kmenem *Salmonella* Enteritidis, PT 8 (wildtype) v množství přibližně 4 log, 2,5 log, 1,5 log a 0,5 log buněk na kus a skladováno ve vybraných modifikovaných atmosférách po dobu 14 dní. Počty salmonel byly stanovovány metodou Most Probable Number (MPN) 0., 3., 7., 10. a 14. den skladování). V průběhu skladování nedošlo v atmosféře 20% CO₂/80% O₂ k významnému nárůstu ani poklesu počtu salmonel. U atmosféry 30% CO₂/70% N₂ byl zaznamenán statisticky významný pokles 10., resp. 14. den skladování, který se však projevil pouze u pokusů s počáteční koncentrací salmonel 4 log a 1,5 log buněk na kus. Prokázali jsme, že i nízké počty *S. Enteritidis* v přítomnosti doprovodné mikroflóry dobře přežívají na povrchu drůbeže při skladování při 3 °C, a to v obou testovaných modifikovaných atmosférách. V případě vhodnějších růstových podmínek při porušení chladírenského řetězce tak mohou i výrobky s nízkým počátečním množstvím salmonel představovat pro konzumenta riziko nákazy.

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