The effect of maintaining the cold chain on the shelf life of poultry products

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Abstract

The microbiological quality of poultry products is dependent on a number of factors. The aim of this paper was to ascertain the influence of maintaining the cold chain on microbiological changes in three types of poultry products and their shelf lives. The samples were collected and tested on the day of production and on the day of consumption. While the control group was stored under defined conditions (4 °C) and an experimental group was acquired from the commercial network on the day of consumption. Indicator microflora (the total viable counts, coliform bacteria, *E. coli*, psychrotrophic microorganisms and lactic acid bacteria) along with the occurrence of pathogenic microorganisms (*Salmonella* spp., *Campylobacter* spp., *Listeria monocytogenes* and *Clostridium perfringens)* were monitored. Based on the results obtained, it can be stated that the cold chain is often disrupted during distribution, which leads to an increase in the concentration of microorganisms and sensory changes in the product before the consumption expiry date. In the samples tested no *Salmonella* type bacteria or *L. monocytogenes* were discovered. However, throughout the storage period *Campylobacter* spp. was detected in all of the samples.

L. monocytogenes, *Campylobacter* spp*., Clostridium perfringens*, CPM

Introduction

Recent decades have seen significant changes in the lifestyles of populations, particularly in developing countries. Although cooking has become a fashionable trend, in most cases it is a hobby which is carried out during one's free time - something which most of working people lack. This has led to an increased interest in ready-to-cook products, whose indisputable advantages include the short time required for preparation and cooking. Chicken has a whole range of advantages – apart from the price these include its simple, various heat treatment, low energy value and relatively low fat content. However, unlike other types of meat, poultry contains more water and due to its composition there is no production of lactic acid or decrease in pH after slaughter, as it is in the meat of larger slaughter animals. Poultry offal (particularly the liver and heart) are often contaminated with microorganisms during evisceration and subsequent handling. That is why poultry does not keep for as long as pork or beef under the same hygiene and refrigeration conditions are maintained. In spite of this, there is an effort on the part of the processor to provide as long a shelf life as possible for the consumer. Therefore, the delivery of safe, high-quality poultry products to the retail market is conditional not only on the high quality of the raw materials and their minimal microbiological contamination, but also on the maintenance of cold chain conditions with minimal fluctuations in temperature during transport from the producer, distribution in the retail chain and at the final consumer. The aim of the experiment was to evaluate the influence of the handling and maintenance of cold chain conditions on the shelf life, quality and safety of selected types of ready-to-cook poultry products.

Materials and methods

From the wide range of products on the market, we chose to analyse portioned chicken (chicken hindquarters), chicken in marinade and chicken liver with herbs. The samples were divided into three groups. The first group (FG) was made up of samples collected from the producer on the day of production, which were immediately

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analysed to determine the initial level of bacterial contamination. Samples from the second (control) group (CG) were collected from the producer on the day of production and stored until the expiry date under controlled conditions (at a stable 4 °C temperature) in the cold storage facilities of the Department of Meat Hygiene and Technology at University of Veterinary and Pharmaceutical Sciences Brno; the samples were analysed on the expiry date. Samples from the third (experimental) group (EG) were collected from the retail market (from various chain stores) on the expiry date and then analysed immediately. In each of the groups identified above, 12 consumer packages of the individual products were tested, and 2 samples were collected from each package. All of the samples were analysed for the total viable counts of microorganisms (TVC) as well as the number of psychrotrophic bacteria, coliform bacteria, *Escherichia coli,* and, from pathogenic microorganisms, bacteria of the genus *Campylobacter* spp., *Salmonella* spp., *Listeria* spp. and *Clostridium perfringens*.

The cultivation of the microorganisms collected was carried out under the appropriate ČSN ISO norms: determining the total viable counts (ČSN EN ISO 4833), determining the number of psychrotrophic microorganisms (ČSN ISO 17410), determining the number of mesophilic lactic acid bacteria (ČSN ISO 15214). When determining the number of *Escherichia coli* and coliform bacteria, the chromogenic substrate Coliform agar ES was used (Merck, Germany). The cultivation was carried out using a suffusion technique: the samples were incubated at 37°C for 22 ± 2 hrs. The presence of pathogenic microorganisms in the samples was also determined: the detection of thermotolerant species of the genus *Campylobacter* (ČSN ISO 10272), determining the number of *Clostridium perfringens* (ČSN EN ISO 7937), the horizontal method for the detection of *Salmonella* spp. (ČSN EN ISO 6579) and the horizontal method for the detection of *Listeria monocytogenes* (ČSN EN ISO 11290). Suspected pathogen isolates (*Campylobacter* spp., *Listeria* spp., *Clostridium perfringens*) were confirmed based on the appropriate norms and also using the methods of molecular biology mPCR, PCR/RFLP (Denis 1999; Baums 2004; Huang 2007). The results obtained from the individual findings were converted into logarithmic values. The numbers of microorganisms found in the control and experimental groups at the end of the shelf life were compared using an unpaired Student t-test in the Statistica 7.1 programme (StatSoft Inc., USA). The value $P < 0.05$ was considered statistically significant.

Results and discussion

When comparing the results of the observed concentrations of microorganisms for the individual groups, statistically significant differences were discovered in the values measured in the samples acquired from the commercial outlets (the experimental group) and the samples stored at a temperature of 4 °C (the control group). The results were also different for the individual types of chicken products tested. Initial concentration of all the observed microorganisms found was the greatest in the chicken in marinade, whilst the lowest was in the chicken liver with herbs.

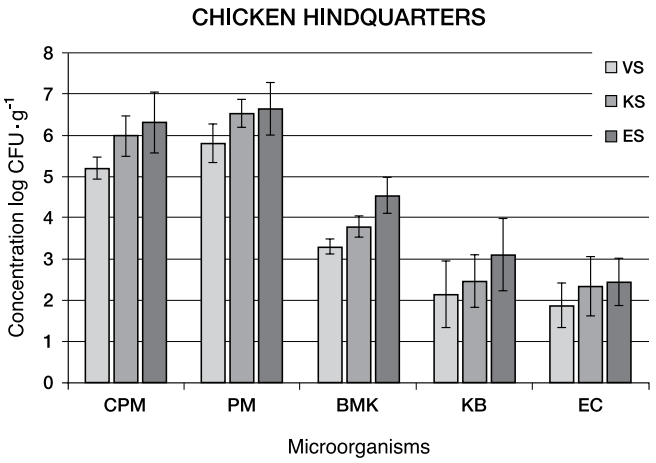

Fig. 1. Concentration of observed microorganisms for the default group (VS), control group (KS) and experimental group (ES) in samples from the chicken quarters (CPM -total viable counts, PM – psychrotrophic microorganisms, BMK – lactic acid bacteria, KB – coliform bacteria, EC – *E. coli*)

As expected, the lowest values for the indicator microorganisms (TVC, coliform microorganisms and *E. coli*) were detected on the day of production. When storing the products, both in the controlled temperature regime (4 °C) and under the distribution conditions of the commercial network, the result was an increase in the number of all the groups of microorganisms tested was observed under observation. For the chicken hindquarters (Fig. 1) statistically significant differences ($P < 0.001$) in lactic acid bacteria were determined compared with the control and experimental groups.

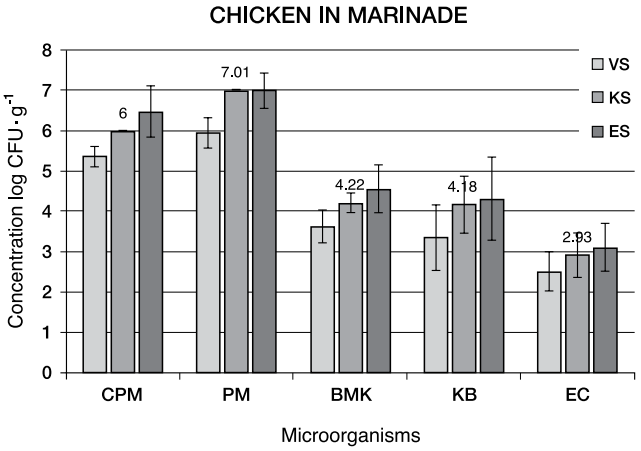

Fig. 2. Concentration of observed microorganisms for the default group (VS), control group (KS) and experimental group (ES) in samples from the chicken quarters (CPM -total viable counts, PM – psychrotrophic microorganisms, BMK – lactic acid bacteria, KB – coliform bacteria, EC – *E. coli*).

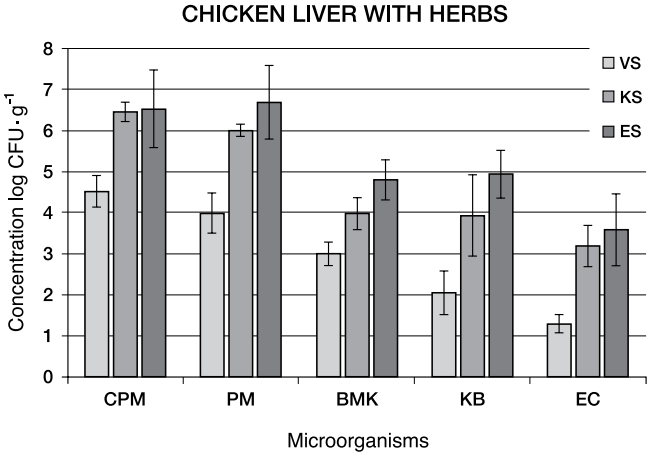

Fig. 3. Concentration of observed microorganisms for the default group (VS), control group (KS) and experimental group (ES) in samples from the chicken quarters (CPM -total viable counts, PM – psychrotrophic microorganisms, BMK – lactic acid bacteria, KB – coliform bacteria, EC – *E. coli*)

For the chicken in marinade (Fig. 2), the initial microbial contamination was the highest of all the products under observation, and the differences in the total number of microorganisms between this group and the control and experimental groups were statistically significant ($P = 0.02$). For the chicken liver with herbs (Fig. 3), there was a significant increase in the microorganisms under observation, with higher numbers again shown for the samples obtained from the market network; statistically significant differences between the control and the experimental groups were also found in the incidence of lactic acid bacteria ($P < 0.001$), psychrotrophic microorganisms ($P = 0.02$) and coliform microorganisms ($P = 0.006$). For this product (chicken liver with herbs), significant sensory changes were detected in colour (greening) and in smell (a putrid smell) in the experimental group at the end of the shelf life, whilst in contrary to the control group where no sensory changes were recorded registered.

The differences in the microbial status between the product types under observation are influenced both by the primary microbial contamination and by the type of product and means of packaging. The reason for the high microbial contamination of the chicken in marinade is probably the increased handling of the raw material when dividing it into pieces and the use of spices, in this case marinade spices, which may represent cause a microbial contamination. An important role is also played by the quality and age of the raw materials used. Some producers and retailers often try to "salvage" raw material which is just before its expiry date. The salt content and low pH of the marinade slows down the multiplication of microorganisms and thus extends the shelf life. The largest number of lactic acid bacteria was found in chicken pieces in marinade, and this is probably related to the method of packaging used: a higher incidence of bacteria is commonly found in vacuum-packed products. Similarly, high numbers of lactic acid bacteria in chicken liver with herbs may be caused by the use of protective atmosphere packaging. Lactic acid bacteria are a natural part of the saprophytic microorganisms of meat, and when meat is packaged in reduced oxygen packaging (vacuum packaging or a majority of protective atmosphere systems) these develop and often become the dominant element of the microflora.

Salmonella spp. and *Listeria monocytogenes* as representatives of pathogenic microorganisms, were not detected in any of the products, which demonstrates the high level of hygiene in food premises during the acquiring and processing of raw materials and compliance with all of the HACCP rules.

However, in all of the different products, non-pathogenic species of *Listeria welshimeri* (8 isolates) and *Listeria innocua* (13 isolates) were found. The highest level of contamination was found in the chicken liver with herbs. The incidence of non-pathogenic microorganisms indicates the ubiquitous nature of Listerias.

Clostridium perfringens was only found in the samples from the chicken quarters and the marinated chicken in the experimental group, and only in the samples from the end of the shelf life. Fourteen isolates of *C. perfringens* were obtained from all of the samples and these were classified as type A by multiplex PCR, but molecular biology methods failed to demonstrate the presence of the enterotoxin gene. Enterotoxigenic strains of *C. perfringens* are the cause of foodborne disease in humans, though their prevalence is only around 5%. The most often isolated type in foodstuffs is type A, which is a normal part of the digestive tract of both animals and humans. The pathogenic action of *C. perfringens* is dependent on the target tissue. Alongside its other biological activity, the alpha toxin type A causes an increase in vascular permeability and haemolysis, swelling and skin necroses. The presence of *Clostridium* spp. in poultry products can thus serve as an indicator of level of hygiene (Fazil et al. 2002).

An important result is the positive detection of thermophilic campylobacters in all of the analyzed products throughout the shelf life. The most frequently demonstrated types were *C. jejuni* (15%) and *C. coli* (25%) and more than half of the samples showed a mixed

presence of both of this species (i.e. *C. jejuni* and *C. coli* in one sample). The incidence of thermophilic campylobacters was recorded both on the day of production as well as at the end of the shelf life in both the control and the experimental all samples groups. The discovery of *Campylobacter* spp. can be viewed as both is not only very interesting and but at the same time alarming. Thermophilic campylobacters are generally considered to be microorganisms which multiply in temperatures over 30 °C and are very sensitive to low pH, low water activity, the presence of NaCl and oxygen. From the literature it is known that under adverse conditions *Campylobacter* spp. survives only 2-4 days in *in vitro* laboratory experiments (Nguyen et al. 2006). Nevertheless, many technological studies have shown the positive detection of this pathogen in minced poultry meat after 14 days (Sampers et al. 2010), with Nneither low temperatures of around 4 °C nor a concentration of NaCl around 1.5% having had any negative effect on its survival (Harrington et al. 2007; Habib et al. 2008). The products under observation tested in our study were stored at cold-storage temperatures which prevent the multiplication of thermophilic campylobacters; the marinated chicken contained salt, the marinade also had a low pH level and the chicken quarters were only wrapped in simple packaging which does not significantly influence the passage of oxygen. In spite of this, the products were shown to be positive for *Campylobacter* spp. during the entire timeline under observation. The reason for the survival of the pathogen during the products' storage period is probably the high water activity and the low temperature, which slows the devitalisation of cells. At the same time, certain products (marinated chicken and chicken liver with herbs) had lower oxygen content due to the method way of packaging, which can positively influence the lifespan of campylobacters.

Legislative requirements for the microbiological safety of foodstuffs have been set out by the Commission Regulation (EC) 1441/2007 on microbiological criteria for foodstuffs. For the sale of meat and meat products there is only one requirement during their shelf life – the absence of *Salmonella* spp. This requirement was met by all the samples analysed. In addition to this legislative provision, producers can also make use of ČSN 56 9609 for establishing microbiological criteria in food production. For packaged cuts of meat it is recommended that the concentration of *E. coli* and coagulase-positive staphylococci is measured and that the absence of *Salmonella* spp. is also demonstrated. The maximum *E. coli* concentration allowed is log 2.7 $CFU \cdot g^{-1}$. In our study, the average concentration of *E. coli* met the recommended criteria in all cases. For chilled meat products, the monitoring of the same groups of microorganisms and also sulphate-reducing clostridia is recommended. The same maximum concentration applies for both *E. coli* and sulphate-reducing clostridia, i.e. log 4 $CFU \cdot g^{-1}$. The recommended concentrations were observed in all samples and, from the viewpoint of health risks due to the handling of raw meat and meat products, the products analysed in this study fulfilled the food safety criteria.

Conclusions

Producers should bear in mind that a product's established expiry date is safe as long as the product is prepared from high-quality raw materials with minimum primary microbial contamination, as long as the HACCP system is operating properly during its production, and particularly as long as proper conditions are observed during transportation and subsequent distribution in the retail. Otherwise, microbial contamination can lead to the spoilage of the poultry product before the end of its shelf life, which could cause foodborne illness. It is clear that even short-term fluctuations in the cold chain lead to significant microbial changes and as a consequence undesirable sensory changes to the product. The discovery of pathogenic microorganisms, in particular *Campylobacter* spp., is alarming, especially for the target customer. Uncooked poultry and poultry products should be considered as

potentially hazardous. It is necessary to store them according to the producer's instructions and to comply with basic hygiene standards when handling them, and in particular to observe procedures for the thorough heat treatment of food prior to consumption.

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