Quality and microbiological stability of chilled chicken legs treated with lysozyme

J. KIJOWSKI*, C. MARCISZEWSKA and A. POPIÓŁ

Department of Food Quality Management, A. Cieszkowski Agricultural University, 60-624 Poznań, Poland

* kijowski@au.poznan.pl

Keywords: chicken legs; cold storage; lysozyme; shelf-life; sensory quality

Summary
The study investigated the effect of spraying chicken legs with skin with lysozyme solutions of varying activity on their microbiological stability and organoleptic features. Lysozyme was applied at concentrations ranging from 3000 to 48000 U/ml. The effect of storage time at the temperature of +4°C on the total aerobic bacterial count, coli titre, the occurrence of enterococci, anaerobic sporeforming bacilli and pathogenic staphylococci was analyzed, along with the examination of sensory quality attributes. The investigations showed that the addition of lysozyme resulted in a considerable inhibition of growth of the initial aerobic bacterial counts and a limitation of disadvantageous organoleptic changes during cold storage of samples. Lysozyme solution with the activity of 48000 U/ml caused a 20-fold reduction in the initial aerobic bacterial count. Sensory examination showed that samples subjected to the action of lysozyme and stored for 120 h under cold storage conditions did not differ qualitatively from fresh elements. The obtained results showed that lysozyme might be an effective agent extending shelf-life of portioned poultry meat.

Introduction
The dynamic development of trade and distribution channels along with the increasing demand for portioned, non-frozen meat encourage producers to constantly improve methods of cold storage of fresh meat, aiming at the high quality, wholesomeness, safety and increasingly long shelf-life of meat (Jelle, 1991; Kuzia, 1998; Cosby et al., 1999; Kijowski, 1999; Woods and Church, 1999; Kijowski et al., 2002). Poultry meat is offered to consumers in the form of chilled whole carcasses, carcass elements with skin, such as quarters, legs, thighs, wings or skinned and deboned breast fillets.

The microbiological conditions of raw poultry carcasses or meat are often unsatisfactory and for this reason it is attempted to limit the counts of microorganisms, both pathogenic and saprophytic, on its surface. It is made possible by the introduction of new carcass pre-washing decontamination methods, such as the addition of chlorine-releasing agents (e.g. sodium oxochlorate) to carcass water washing systems, washing carcasses with alkaline trisodium phosphate added, the ozonization of chilling water, the addition of lactic acid and its salts, or the application of ionizing radiation. Novel methods of extending shelf-life in foodstuffs consist in the application of antibacterial agents called bacteriocins, as well as the use of lysozyme, an enzyme with bacteriostatic properties, naturally occurring in chicken egg white (Kijowski and Lesnierski, 1995; Kijowski et al., 2002). Lysozyme isolated from egg white exhibits bacteriostatic action not only against saprophytic bacteria, but also against food pathogens such as Listeria monocytogenes, or Clostridium botulinum (Hughey and Johnson, 1987; Payne et al., 1994).

The application of bacteriostatic agents will be possible only in those slaughterhouses and poultry processing plants, in which principles of Good Manufacturing Practice (GMP) and Good Hygienic Practice (GHP) or the HACCP are implemented and system function effectively. Otherwise, such agents would only help to hide shortages in the maintenance of required hygienic standards.

In spite of the fact that the lysozyme monomer has found numerous applications in the preservation of foodstuffs, especially in order to extend shelf-life of fresh food, there are practically no studies on the microbiological quality of poultry meat and the effect of this enzyme on the organoleptic quality of meat treated with lysozyme (Kijowski et al., 2002).

Thus, the aim of this study is to assess the effectiveness of spraying skin surface of chicken legs with a lysozyme solution with a determined activity against saprophytic bacteria causing food spoilage, and to assess sensory quality of poultry elements under cold storage conditions.
Materials and methods

Material was collected at the “EXDROB” Poultry Processing Plant in Kutno. Legs with skin (thighs and drumsticks) of 6-week broiler chickens were used in the experiments. These elements were obtained after the division of carcasses on a Danish automatic line by Linco. Using a lysozyme preparation with the initial activity of 23870 U/mg produced according to the method developed by the authors (Kijowski et al., 2000) at the Laboratory of the Department of Food Quality Management, Agricultural University of Poznań, solutions with varying activity, i.e. 3000, 6000, 12000, 24000, 48000 U/ml were obtained (U= unit of activity). Samples were sprayed with lysozyme solutions until their whole surface was uniformly covered, and subsequently packed on styrofoam trays with an absorbent liner and covered with an LMA-350 polyethylene permeable unshrinkable film. The interval between the slaughter of chickens and the beginning of the first tests was approx. 3 hours. Microbiological quality assessment of chicken legs included total aerobic bacterial counts, coli titre, the presence of enterococci, anaerobic sporeforming bacilli, pathogenic staphylococci, as well as the presence of Salmonella. Microbiological analyses were performed using analytical procedures (Maleszewski, 1981; Burbianka and Pliszka, 1983; Duszkiewicz-Reinhard et al., 1999).

Changes in the total microbial counts during storage were described in the time-effect system, where effect was always calculated as a relative value in the form of the $N_t/N_0$ ratio, i.e. the proportion of the microbial count in meat after a given time $t$ to the microbial count in meat immediately after the production process.

To describe the changes in the effect, a logarithmic function was applied of the form:

$$\ln(N_t/N_0) = \ln A + b \times t$$

where:
- $N_t$ – microbiological contamination after storage time $t$,
- $N_0$ – initial microbiological contamination of meat immediately after the production process for storage time $t=0$,
- $A$ – effect calculated for storage time $t=0$, i.e. at the beginning of the experiment,
- $t$ – storage time (hours),
- $b$ – slope of a straight line, characterizing the dynamics of changes in the total bacterial count in meat subjected to the action of lysozyme after given storage times to the total bacterial counts found in meat subjected to the action of lysozyme with the same activity immediately after the production process.

Sensory examination of meat quality was conducted assessing the appearance, colour, aroma and texture of poultry elements immediately after chilling and after different storage times at the temperature of 4°C. Quality attributes as well as the point score scale were developed on the basis of criteria given in Polish standards (PN-A-86524: 1994; PN-A-86520: 1984; PN-A-82101: 1980). Consumer traits were examined by a 4-person panel according to a 5-point scale. The adopted scale corresponded to five basic quality levels for each quality attribute: the score of 5 points corresponded to very good quality, 4 – good, 3 – satisfactory, 2 - unsatisfactory, while 1 – bad (Baryło-Pikielna, 1975). Moreover, on the basis of the conducted examination of individual quality attributes, i.e. general appearance, colour, aroma, consistency, the quality of samples was evaluated as expressed in the form of one number called the weighted mean. The following weighting coefficients were adopted in the calculations for individual attributes: general appearance - 0.1, colour – 0.3, aroma – 0.4, consistency – 0.2 (PN –80/A-82101). For the purpose of practical inference, storage time limited by the disqualifying score and the score above satisfactory was adopted as the shelf-life criterion. Poultry elements were evaluated approx. 15 min after being taken out of the cooler, in which they were stored at the temperature of 4°C. Sensory examination was conducted until the day the first symptoms of spoilage were observed, especially distinct changes in aroma.

Results and discussion

Previously conducted microbiological analyses of chicken breasts showed that lysozyme activity of 2400 U/ml was only slightly effective (Kijowski et al., 2002). Additionally, poultry skin, due to its structure and it being highly adhesive for bacteria, is as a rule more microbiologically contaminated. For the five examined series, initial (i.e. immediately after slaughter) microbiological contamination of the surface of chicken legs with skin was $2.2 \times 10^5$ cfu/cm². In the five examined sample series in the area of 1 cm² coli titre exceeding 0.01 ml was found only in one case. In three cases the enterococci titre was 0.1ml, while in one it was 0.01ml and in one no enterococci were found in 0.1ml.

The effect of microbial control was already observed after the application of lysozyme with the activity of 3000 U/ml. Initial (after slaughter) microbiological contamination of poultry legs was on
average $7.6 \times 10^4$ cfu/cm$^2$ and it was almost three times lower than the contamination of control samples. The effectiveness of lysozyme application increased proportionally to the enzyme activity. However, during cold storage the effects of initial elimination of bacteria were observed at higher enzyme activities, i.e. 12000 U/ml, and especially at 24000 and 48000 U/ml (Figure 1).

![Figure 1](image1.png)

Figure 1  The effect of lysozyme on changes in counts of aerobic bacteria for chicken legs covered with skin stored at the temperature of 4°C. In chicken leg samples treated with lysozyme with the activity of 6000, 12000, 24000 and 48000 U/ml, the initial microbiological contamination was considerably lower than that in the control samples. The application of lysozyme with the activity of 6000 U/ml resulted in a threefold, while with the activity of 12000U/ml – a fourfold decrease in the initial microbiological contamination. However, in comparison to the reduction of initial contamination observed on breast muscles (20-fold) at the application of lysozyme with the activity of 12000U/ml (Kijowski et al., 2002), it may be concluded that the surface of the skin (higher pH, considerable undulation) is conducive of the development of microorganisms, it exhibits increased adhesiveness and forms a protective layer for microorganisms. Similar conclusions concerning microbiological contamination found on poultry skin may also be found in some other publications (Fehlhaber, 1996; NACMCF, 1997; Tamblyn and Conner, 1997).

The application of lysozyme with the activity of 24000 U/ml reduced initial microbiological contamination 10 times, while that with the activity of 48000 U/ml resulted in an approx. 20-fold reduction of the bacterial count.

Changes in the total bacterial count during storage, also described in the time-effect system, indicate a distinct limitation of microbial growth by lysozyme (Figure 2).

![Figure 2](image2.png)

Figure 2  The effect of lysozyme activity on changes in total counts of aerobic bacterial for chicken legs with skin stored at the temperature of 4°C, expressed in the semi-logarithmic scale ln(N/N$\circ$).
After 72 h storage microbiological contamination in samples with the addition of lysozyme with the activity of 12000 U/ml was identical to that in samples with no lysozyme added on the production day. Such an effect was possible due to the lowering of the initial bacterial cell counts. This contamination increased in the course of storage, although its dynamics in case of chicken legs treated with lysozyme was considerably lower, as shown by the slopes of curves (Figure 2).

After 144 h storage microbiological contamination of leg skin in samples sprayed with lysozyme with the activity of 3000 U/ml and 6000 U/ml was similar to that of control samples, which confirms the fact that skin surface is conducive of the development of microorganisms. It is indicated by the fact that in the previous experiment on chicken breasts an effective limitation of bacterial growth during cold storage of samples was already shown at the enzyme activity of 6000 U/ml (Kijowski et al., 2002).

The application of lysozyme with the activity of 12000 U/ml on the skin surface of poultry legs after 144 h cold storage in comparison to the control samples after identical storage time resulted in microbial contamination being 6 times lower, whereas for samples with the addition of lysozyme with the activity of 24 000 U/ml, it caused an over 20-fold decrease in the contamination. Initial microbial contamination, as well as the dynamics of microbial growth on the skin surface during cold storage at the temperature of 4°C were comparable for samples treated with lysozyme with the activity of 24 000 U/ml and 48 000 U/ml.

Coli titre was presented for the surface of 1 cm² skin and amounted on the day of production to 0.01 for samples with no lysozyme added, and only in one case it was 0.001. During cold storage of these meat samples coli titre increased considerably, as after 120 and 144 h storage for individual series it was 0.001 or 0.0001 and above these values, and only in one case it was 0.01. In samples treated with lysozyme solutions coli titre not only was not increasing, but also no growth of coli bacteria was observed. In the course of storage at lysozyme activity of 6000 U/ml coli titre remained at the level of 0.1 or 0.01 and only in one case it was 0.001 after 144 h storage. In meat samples with lysozyme added at activities of 12000, 24000 and 48000 U/ml after 144 h storage coli titre was 0.1 and only in one case it was 0.01.

Assuming that poultry meat meets Polish microbiological standards if coli titre on the surface of 1 cm² does not exceed 0.01, it may be stated that for control samples on the day of production this requirement was met in 80%, in samples treated with lysozyme with activities of 6000 U/ml and higher in 100% (coli titre was 0.1 or 0.01). The fraction of chicken legs meeting the standards in 100% after 144 h storage consisted of samples treated with lysozyme solutions with the activities of 12000, 24000 and 48000 U/ml (Figure 4). In poultry leg samples, which were not sprayed with lysozyme, enterococci were detected on the skin. On the day of production in five tested series on the surface of 1 cm² skin of poultry legs enterococci were detected in three cases at 0.1 ml, in one case at 0.01 ml, and only in one case these bacteria were not found. After 144 h storage enterococci were not detected only in case of one sample, in one sample they were found at 0.1 ml, in two samples they were detected at 0.01 ml and in one case – at 0.001 ml. In turn, on the surface of 1 cm² skin of legs treated with lysozyme solutions no enterococci were detected at 0.1 ml or they were found at 0.1 ml already at the activity of 3000 U/ml. The application of lysozyme with the activity of 24000 U/ml resulted in the inhibition of growth of enterococci. It was found that in two out of the five tested samples enterococci were present at 0.1 ml. When spraying with lysozyme with the activity of 48000 U/ml was applied, an inhibition of growth was observed on the skin of chicken legs in case of enterococci both at the beginning of storage and after 144 h cold storage.

Metcalf and Deibel (1973) in their studies also showed a bacteriostatic action of lysozyme on enterococci.

In the investigated samples no pathogenic staphylococci were found in 0.1 g and anaerobic sporeforming bacilli in 0.1 g.

On the other hand, Salmonella enteritidis bacteria were detected both in samples treated with lysozyme and controls. The conducted tests confirm previous reports that the presence of lysozyme has no inhibitory effect on the growth of these bacteria.
While analyzing sensory attributes of control samples of chicken legs with skin stored under cold storage conditions for 144 h, a deterioration of all the evaluated parameters was observed. A change was found in the colour of leg skin, which was darker, especially on cutting lines and muscles showed through the skin. Meat exhibited weaker springiness and the aroma intrinsic to fresh meat was practically undetectable. Muscles of chicken legs, of which the skin was sprayed with a lysozyme solution with the activity of 6000, 12000, 24000 or 48000 U/ml, and 120 h storage at the temperature of 4°C, did not differ in terms of sensory attributes from fresh samples. After this duration of storage the lowest score was given for aroma, which for samples treated with lysozyme with the activities of 6000 and 12000 U/ml received notes above satisfactory. Aroma was slightly changed, but difficult to define; however, it was not intrinsic to fresh poultry meat. A similar note was given for consistency — poultry leg muscles exhibited lower springiness, at pressure they were permanently deformed. At the same time of storage control samples received 2 points each for aroma and consistency, which corresponded to an unsatisfactory quality level. Aroma was the most affected parameter, as it was defined as a slightly putrid off-odour. The outer surface was slimy, sticky, the colour was changed into green in some places, defined also as sallow, muscles showed through the skin and the muscle tissue at exerted pressure was loose and deformed easily. Sensory features of legs with skin, sprayed with lysozyme solutions with the activity of 6000, 12000, 24000 and 48000 U/ml, after 144 h storage were slightly worse than those after 120 hours. Aroma and consistency deteriorated further, as aroma was less intrinsic to fresh poultry meat, slightly changed, but difficult to define. Consistency became looser and for this reason it was evaluated as being between good and satisfactory. Dark red muscles showed through the skin, on the inside the muscles were light, light-cream in colour. The surface was slightly moist; the overall appearance was not very objectionable. Also in this case the deterioration of sensory attributes was always accompanied by an increase in the total bacterial counts, coli titre and counts of enterococci.

On the basis of the conducted sensory examination of individual quality attributes, a full evaluation of product quality was carried out and it was presented in the form of one number, the weighted mean (Figure 4). Overall sensory examination showed that poultry legs stored for 120 h under cold storage conditions, treated with lysozyme with the activity of 6000 U/ml received 3.75 points, whereas in case of the activity of 12000 U/ml – 3.95 points, which corresponded to satisfactory quality of the product. Samples treated with lysozyme solutions with the activity of 24000 and 48000 U/ml after 120 h cold storage received 4.3 points each, which corresponded to good product quality. After 144 h cold storage samples treated with lysozyme with the activity of 24000 and 48000 U/ml received 4 points each, which corresponded to good product quality. In contrast, control samples and samples treated...
with lysozyme with the activity of 3000 U/ml after identical storage times received notes below 3 points, which was equivalent to unsatisfactory quality, which further deteriorated after 144 h storage.

**Figure 4** Weighted mean of overall sensory examination of chicken legs with skin treated with lysozyme and stored at the temperature of 4°C.

### Conclusions

1. Spraying the surface of chicken legs, chilled after slaughter to the temperature of 4°C, with a lysozyme solution results in a significant decrease in total microbial counts. At the activity of 48000 U/ml of lysozyme solution a 20-fold reduction in the initial microbial count was observed.

2. Chicken legs treated with lysozyme and subsequently stored for 6 days at the temperature of 4°C showed a significant inhibition of the dynamics of microbial growth in comparison to control samples. Microbiological contamination on the skin surface of legs treated with lysozyme with the activity of 24000 U/ml after 120 h storage was comparable to the contamination of control samples on the day of production.

3. Lysozyme showed inhibitory action against coli form bacteria on the day of its application and this effect was maintained for as long as 6 days of cold storage of chicken legs. Lysozyme with the activity of 48000 U/ml inhibited the growth of enterococci after its application and during 6 days of cold storage.

4. Sensory examination of chicken legs treated with lysozyme solution with the activity of at least 6000 U/ml after 5 days of storage at 4°C gave results similar to the quality of fresh elements on the day of slaughter. After such storage time in case of control samples condemning sensory changes were observed.

### References


