

EFFECT OF LYSOZYME TREATMENT ON QUALITY AND BACTERIAL CONTAMINATION OF CHILLED CHICKEN LEGS

JACEK KIJOWSKI, CELINA MARCISZEWSKA¹, RENATA CEGIELSKA-RADZIEJEWSKA,
AND ANNA POPIÓŁ²

Department of Food Quality Management,
Poznan University of Life Sciences, 60-624 Poznan, Poland,
¹Sanitary and Epidemiology Station, 99-300 Kutno, Poland
²Poultry Slaughterhouse J. Chlebowska, 64-212 Nieborza, Poland
kijowski@up.poznan.pl

Received: October 9, 2012

Accepted: February 13, 2013

Abstract

The effect of spraying lysozyme solutions of varying activity on microbiological stability and organoleptic features of chicken legs with skin was investigated. Lysozyme was applied at concentrations ranging from 3,000 to 48,000 U/mL. The effect of storage time at 4°C on the total aerobic bacterial count, coli titre, occurrence of enterococci, anaerobic spore forming bacilli, and pathogenic staphylococci was analysed 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 the legs. The lysozyme solution with the activity of 48,000 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 legs. The obtained results revealed that lysozyme might be an effective agent extending shelf-life of portioned poultry meat.

Key words: chicken legs, cold storage, lysozyme, bacteriological flora, shelf-life, sensory quality.

The dynamic development of trade and distribution channels along with the increasing demand for portioned, non-frozen meat encourage producers to the constant improvement of methods of cold storage of fresh meat, aiming at the high quality, wholesomeness, safety, and increasingly its long shelf-life (4, 9, 10, 14, 30). Poultry meat is offered to consumers in the form of chilled whole carcasses, carcass elements with skin, such as quarters, legs, thighs, and wings, or skinned and deboned breast fillets.

The microbiological status of raw poultry carcasses or meat is often unsatisfactory and for this reason it is attempted to limit the counts of microorganisms, both pathogenic and saprophytic, on meat surface. It is possible by the introduction of new carcass pre-washing decontamination methods, such as an addition of chlorine-releasing agents (*e.g.* sodium oxochlorate) to carcass water washing systems, washing carcasses with alkaline trisodium phosphate added, ozonisation of chilling water, an addition of lactic acid and its salts, or an application of ionising radiation. Novel methods of extending shelf-life in foodstuffs consist of application of antibacterial agents called bacteriocins, as well as the use of lysozyme, an enzyme with bacteriostatic properties, naturally occurring in chicken egg white (12, 13). 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* (8, 22).

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 HACCP are implemented and the system functions 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 the enzyme on the organoleptic quality of meat treated with lysozyme (13).

Thus, the aim of the study was 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 most important pathogens, and to assess sensory quality of poultry elements under cold storage conditions.

Material and Methods

The material for analyses was collected at the poultry processing plant. Skin-on legs (thighs and drumsticks) of 6-week broiler chickens were used. These elements were obtained after the division of carcasses on a Danish automatic line by Linco. Lysozyme solutions of varying activity, *i.e.* 3,000, 6,000, 12,000, 24,000, and 48,000 U/mL were obtained (U - unit of activity) from a lysozyme preparation with the initial activity of 23,870 U/mg, produced according to the method developed by the authors (12). The legs 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 the chickens and the beginning of the first tests was approx. 3 h.

Microbiological quality assessment of the legs included total aerobic bacterial counts, coli titre, the presence of enterococci, anaerobic spore forming bacilli, pathogenic staphylococci, as well as the presence of *Salmonella* organisms. Microbiological analyses were performed using analytical procedures, according to the Polish Standards (2, 5, 15, 23, 24). The presence of enterococci was determined using crystal violet azide broth. Incubation was performed at 37°C for 48 h. Giolitti-Cantoni broth and Baird-Parker agar were used to determine the presence of staphylococci. Incubation was carried out at 37°C for 48 h. Coli titre was determined using Brilliant Green Bile Broth medium and Endo agar and incubation was performed at 37°C for 48 h. The presence of anaerobic spore forming bacilli was determined in Wrzosek's medium. Incubation was performed at 37°C for 48 h.

Changes in total microbial counts during storage were described in the time-effect system, where the 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, namely:

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

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 (h),

b – slope of a straight line, characterising 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 immediately after chilling and after different storage times at 4°C. Quality attributes, as well as the point score scale were developed on the basis of criteria given in respective Polish standards (25-27). 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 (1). Moreover, on the basis of the conducted examination of individual quality attributes, *i.e.* general appearance, colour, aroma, and consistency, the quality of samples was evaluated and expressed in the form of one number called the weighted mean. The following weighting coefficients were adopted in the calculations of individual attributes: general appearance – 0.1, colour – 0.3, aroma – 0.4, and consistency – 0.2 (26). 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 4°C. Sensory examination was conducted until the day the first symptoms of spoilage were observed, especially distinct changes in aroma.

For the control sample and samples with varying activities of lysozyme, five series were performed and four samples were examined in each series.

The values of means were analysed using descriptive statistics, *i.e.* standard deviations and a 95% confidence interval. Parameters of exponential models were calculated applying the SPSS PC+ programme.

Results

For the five examined series, initial (*i.e.* immediately after slaughter) microbiological contamination of the surface of skin-on chicken legs was 2.2×10^5 cfu/cm². In five examined sample series in the area of 1 cm², a coli titre exceeding 0.01 ml was only found in one case. In three cases the enterococci titre was 0.1 ml, while in one it was 0.01 ml and in one no enterococci were found in 0.1 ml.

The positive effect of microbial control was already observed after the application of lysozyme with the activity of 3,000 U/mL. Initial (after slaughter) microbiological contamination of poultry legs was on average 7.6×10^4 cfu/cm² and it was almost three times lower than the contamination of control samples. The effectiveness of lysozyme application increased in proportion to the enzyme activity. However, during cold storage, the effects of initial elimination of bacteria were observed at higher enzyme activities, *i.e.* 12,000 U/mL, and especially at 24,000 and 48,000 U/mL (Fig. 1).

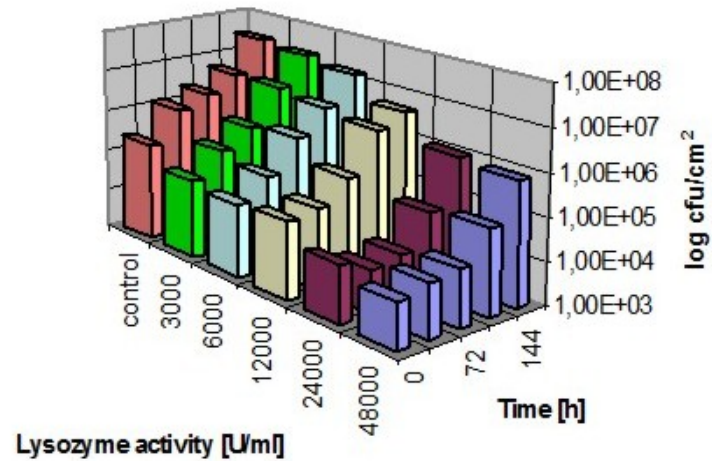


Fig. 1. The effect of lysozyme on the counts of aerobic bacteria for skin-on chicken legs stored at 4°C

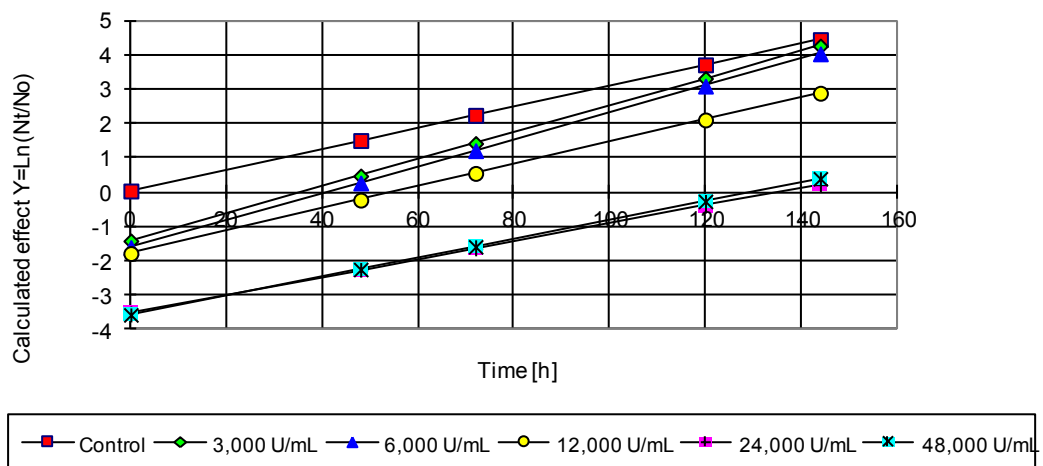


Fig. 2. The effect of lysozyme activity on the total counts of aerobic bacteria for skin-on chicken legs stored at 4°C, expressed in the semi-logarithmic scale $\ln(N_t/N_0)$

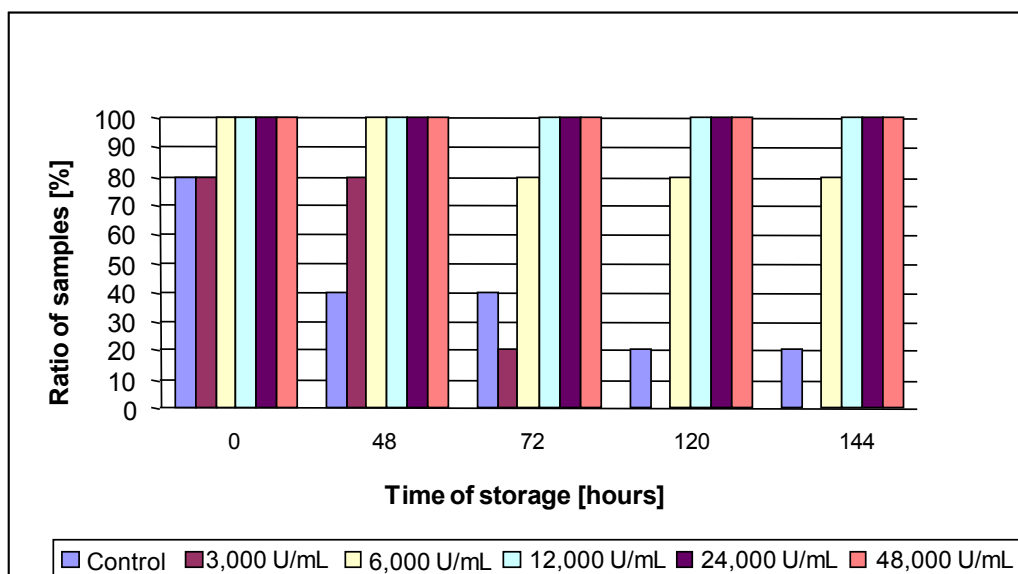


Fig. 3. Percentage of meat samples of skin-on chicken legs, stored at 4°C and treated with lysozyme, for which coli titre did not exceed 0.01

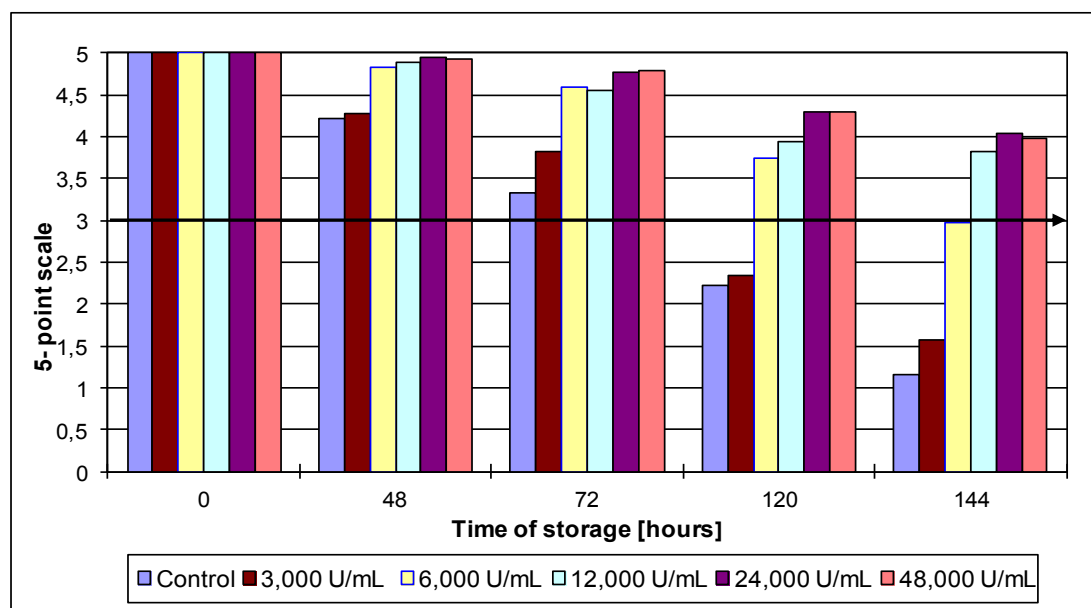


Fig. 4. Weighted means for overall sensory examination of skin-on chicken legs treated with lysozyme and stored at 4°C

In chicken leg samples treated with lysozyme with the activity of 6,000, 12,000, 24,000, and 48,000 U/mL, the initial microbiological contamination was considerably lower than that in the control samples. The application of lysozyme with the activity of 6,000 U/mL resulted in a threefold, while with the activity of 12,000 U/mL – a fourfold decrease in the initial microbiological contamination. The application of lysozyme with the activity of 24,000 U/mL reduced initial microbiological contamination 10 times, while that with the activity of 48,000 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, indicated a distinct limitation of microbial growth by lysozyme (Fig. 2).

After 72 h storage, microbiological contamination of samples with the addition of lysozyme with the activity of 12,000 U/mL was identical to that in samples without 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 (Fig. 2).

After 144 h storage, microbiological contamination of legs sprayed with lysozyme with the activity of 3,000 and 6,000 U/mL was similar to that of control samples. The application of lysozyme with the activity of 12,000 U/mL after 144 h cold storage in comparison to the control samples after an 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. The initial microbiological contamination, as well as the dynamics of

microbial growth on the skin surface during cold storage at 4°C, was comparable for samples treated with lysozyme with the activity of 24,000 and 48,000 U/mL.

Coli titre presented for the surface of 1 cm² of skin amounted to 0.01 on the day of production for samples with no lysozyme added, and only in one case it was 0.001. During cold storage, coli titre increased considerably, as after 120 and 144 h storage it was, for individual series, 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 did not increase and no growth of coliform bacteria was observed. During storage at lysozyme activity of 6,000 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 12,000, 24,000, and 48,000 U/mL after 144 h storage, coli titre was 0.1 and only in one case it was 0.01.

In 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 of skin, 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 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 of skin treated with lysozyme solutions no enterococci were detected at 0.1 ml or they were found at 0.1 ml only in samples with lysozyme added at activities of 3,000 U/mL. The application of lysozyme with the activity of 24,000 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 48,000 U/mL was applied, an inhibition of enterococci growth was observed on the

skin at the beginning of storage and after 144 h cold storage. In the investigated samples no pathogenic staphylococci were found in 0.1 g and anaerobic sporeforming bacilli in 0.1 g.

During the analysis of sensory attributes of control samples of chicken legs 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 were visible through the skin. Meat exhibited weaker springiness and the aroma intrinsic to fresh meat was practically undetectable. Muscles of legs sprayed with a lysozyme solution with the activity of 6,000, 12,000, 24,000, or 48,000 U/mL, after 120 h storage at 4°C did not differ in terms of sensory attributes from the fresh samples. After such storage time, the lowest score was given for aroma which received notes above satisfactory for samples treated with lysozyme with the activities of 6,000 and 12,000 U/mL. Aroma was slightly changed, but difficult to define; however, it was not intrinsic to fresh poultry meat. A similar note was given for the 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, green in places, defined also as fallow, muscles were seen through the skin and the muscle tissue was loose and deformed easily at exerted pressure. Sensory attributes of legs sprayed with lysozyme solutions with the activity of 6,000, 12,000, 24,000, and 48,000 U/mL, were slightly worse after 144 h storage than those after 120 h storage. Aroma and consistency deteriorated further, as aroma was less intrinsic to fresh poultry meat, slightly changed, but difficult to define. The consistency became looser and for this reason it was evaluated as being between good and satisfactory. Dark red muscles were seen through the skin, on the inside, the muscles were light, light-cream in colour. The surface was slightly moist; but the overall appearance was not objectionable. In this case, the deterioration of sensory attributes was always accompanied by an increase in total bacterial counts, coli titre, and counts of enterococci.

On the basis of the conducted sensory examination of individual quality attributes, a complete evaluation of product quality was carried out and it was presented in the form of one number, the weighted mean (Fig. 4). Overall sensory examination showed that poultry legs stored for 120 h under cold storage conditions, treated with lysozyme with the activity of 6,000 U/mL received 3.75 points, whereas in case of the activity of 12,000 U/mL – 3.95 points, which corresponded to satisfactory quality of the product. Samples treated with lysozyme solutions with the activity of 24,000 and 48,000 U/mL received 4.3 points each after 120 h cold storage, which corresponded to good product quality. After 144 h of cold storage, samples treated with lysozyme with the activity of

24,000 and 48,000 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 3,000 U/mL after the same storage times received notes below 3 points.

Discussion

Data indicate that an effective action of many compounds exhibiting antibacterial effects is frequently observed only under model conditions (31). Studies conducted up till now on the antibacterial action of lysozyme in combination with other compounds were conducted first of all in broth. There is a limited number of studies determining the effect of enzyme action on microbiological and sensory quality of meat and processed meat products (3, 13, 16, 17, 20, 21, 28).

The results of the study indicate that spraying the surface of chicken legs, chilled after slaughter to 4°C, with a lysozyme solution results in a significant decrease in the total microbial counts. At the activity of 48,000 U/mL of lysozyme solution, a 20-fold reduction in the initial microbial count was observed. Chicken legs treated with lysozyme and subsequently stored for 6 d at 4°C showed a significant inhibition of the dynamics of microbial growth in comparison to control samples. However, in comparison to the reduction of the initial contamination observed on breast muscles (20-fold) after application of lysozyme with the activity of 12,000 U/mL, it may be concluded that the surface of the skin (higher pH, considerable undulation) is more prone to the development of microorganisms, as it exhibits increased adhesiveness and forms a protective layer for microorganisms (13). Similar conclusions concerning microbiological contamination revealed on poultry skin may be also found in other publications (6, 19, 29). In the previous experiment on chicken breasts, an effective limitation of bacterial growth during cold storage of samples was already demonstrated at the enzyme activity of 6,000 U/mL (13).

It was found that lysozyme displayed an inhibitory action against coliform bacteria on the day of its application and this effect was maintained for 6 d of cold storage. 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 their production this requirement was met in 80% in samples treated with lysozyme with activities of 6,000 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 12,000, 24,000, and 48,000 U/mL (Fig. 3). Studies concerning the use of lysozyme in chicken breast muscles also demonstrated an inhibitory action against coliforms on the day of its application and in the investigated period of cold storage (13). It was stated that low temperature and lysozyme may have a bacteriostatic effect on coliforms, although they belong to the group of Gram-negative bacteria.

Lysozyme with the activity of 48,000 U/mL inhibited the growth of enterococci after its application and during 6 d of cold storage. Metcalf and Deibel (18)

also showed a bacteriostatic action of lysozyme on enterococci. It is difficult to determine the effect of lysozyme on pathogenic staphylococci and anaerobic spore forming bacilli as they were not found in the tested samples. *Salmonella* Enteritidis rods were detected both in samples treated with lysozyme and the controls. The conducted tests confirmed previous reports showing that the presence of lysozyme has no inhibitory effect on the growth of these bacteria (8, 22).

Sensory examination of chicken legs treated with lysozyme solution with the activity of at least 6,000 U/mL after 5 d of storage at 4°C gave results similar to the quality of fresh elements on the day of slaughter. After such storage time, condemning sensory changes were observed in control samples. In previous studies, it was also found that the application of lysozyme monomer solution sprayed on the surface of breast muscles significantly prolonged their shelf-life (3, 13). Similar results concerning the applicability of lysozyme in the extension of shelf-life of meat and meat products were obtained by other authors (7, 16, 21). The results indicate that lysozyme might be an effective agent extending shelf-life of portioned chicken carcasses.

References

1. Barylko-Pikielna N.: An outline of sensory examination. WNT, Warszawa, 1975, pp. 307-323.
2. Burbianka M., Pliszka A., Burzyńska H.: Microbiology of food. PZWL, Warszawa, 1983.
3. Cegielska-Radziejewska R., Leśniewski G., Kijowski J., Szablewski T., Zabielski J.: Effects of treatment with lysozyme and its polymers on the microflora and sensory properties of chilled chicken breast muscles. Bull Vet Inst Pulawy 2009, **53**, 455-461.
4. Cosby D.E., Harrison M.A., Toledo R.T.: Vacuum or modified atmosphere packaging and EDTA – nisin treatment to increase poultry product shelf-life. J Appl Poult Res 1999, **8**, 185-190.
5. Duszkievicz-Reinhard W., Grzybowski R., Sobczak E.: Theory and exercises in general and technical microbiology. Edition of SGGW, Warszawa, 1999, pp. 95-106.
6. Fehllhaber K.: Microbiological problems in slaughter poultry. Med Weter 1996, **52**, 758-762.
7. Gill, A.O., Holley R.A.: Inhibition of bacterial growth on ham and bologna by lysozyme, nisin and EDTA. Food Res Int 2000, **33**, 83-91.
8. Hughey V.L., Johnson E.A.: Antimicrobial activity of lysozyme against bacteria involved in food spoilage and foodborne disease. Appl Environ Microbiol 1987, **53**, 2165-2170.
9. Jelle B.: Influence of a selected *Lactobacillus* strain on the shelf-life of fresh poultry meat. Prevention and control of potentially pathogenic microorganisms in poultry and poultry meat processing. Hygienic aspects of processed poultry meat. Proceedings of 13th European Symposium on the Quality of Poultry Meat, Doorwerth, Netherlands, 1991, pp. 41-47.
10. Kijowski J.: Innovations in poultry industry. Food Indust 1999, **53**, 12-16.
11. Kijowski J., Lesniewski G.: The use of lysozyme for food preservation and in medical diagnosis and pharmacology. Biotechnologia 1995, **2**, 130-140.
12. Kijowski J., Lesniewski G., Fabisz-Kijowska A.: Lysozyme polymer formation and functionality of residuals after lysozyme extraction. In: Egg Nutrition and Biotechnology, edited by J.S. Sim, S. Nakai, W. Guenter, CABI Publishing, UK, 2000, pp. 269-286.
13. Kijowski J., Marciszewska C., Cegielska-Radziejewska R.: Quality and microbiological stability of chilled chicken breast muscles treated with a lysozyme solution. Pol J Food Nutr Sci 2002, 11/52, 47-54.
14. Kuzia A.: Novel concepts in packaging of processed meat products. Mięso i Wędliny 1998, **6**, 20-22.
15. Maleszewski J.: Microbiological monitoring of cleanliness of machinery, equipment, workers' hands and air in processing plants of animal origin foodstuffs. Recommendations of the National Veterinary Research Institute in Pulawy, 1981.
16. Malicki, A., Jarmoluk A., Brużewicz S.: Effect of sodium lactate used alone or in combination with lysozyme on the physico-chemical and microbiological properties of steamed sausage stored under refrigeration. Bull Vet Inst Pulawy 2004, **48**, 47-51.
17. Malicki A., Trziszka T., Szpak M., Źródłowska-Danek J.: Studies on the application of lysozyme and sodium acetate to extend shelf life of poultry meat. Med Weter 2010, **66**, 699-701.
18. Metcalf R.H., Deibel R.: Effect of lysozyme on enterococcal viability in low ionic environments. J Bacteriol 1973, **116**, 278-286.
19. NACMCF: National Advisory Committee on Microbiological Criteria for Foods. Generic HACCP Application in broiler slaughter and processing. J Food Protect 1997, **60**, 579-604.
20. Nattress F.M., Baker L.P.: Effects of treatment with lysozyme and nisin on the microflora and sensory properties of commercial pork. Int J Food Microbiol 2003, **85**, 259-267.
21. Nattress F.M., Yost C.K., Baker L.P.: Evaluation of the ability of lysozyme and nisin to control meat spoilage bacteria. Int J Food Microbiol 2001, **70**, 111-119.
22. Payne K.D., Oliver S.P., Davidson P.M.: Comparison of EDTA and apo-lactoferrin with lysozyme on the growth of foodborne pathogenic and spoilage bacteria. J Food Protect 1994, **57**, 62-65.
23. PN-EN ISO 4833:2004/Ap1:2005. Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of microorganisms. Colony-count technique at 30°C.
24. PN-EN ISO 6579:2003. Microbiology of food and animals feeding stuffs - Horizontal method for detection of *Salmonella* spp.
25. Polish Standard PN-A-86524: 1994/ Az2: 2002. Poultry meat in carcass parts.
26. Polish Standard PN-A-82107: 1996. Delicatessen. Organoleptic and physical tests.
27. Polish Standard PN-A-86520: 1998. Poultry products. Poultry carcasses.
28. Rao M.S., Chander R., Sharma A.: Synergistic effect of chitooligosaccharides and lysozyme for meat preservation. Food Sci Technol 2008, **41**, 1995-2001.
29. Tamblyn K.C., Conner D.E.: Bactericidal activity of organic acids against *Salmonella typhimurium* attached to broiler chicken skin. J Food Protect 1997, **60**, 629-633.
30. Woods L.F.J., Church P.N.: Strategies for extending the shelf-life of poultry meat and products. In: Poultry Meat Science, edited by R.I. Richardson, G.C. Mead, CAB International, UK, 1999, pp. 297-311.
31. Zhang H., Kong B., Xiong Y.L., Sun X.: Antimicrobial activities of spice extracts against pathogen and spoilage bacteria in modified atmosphere packaged fresh pork and vacuum packaged ham slices stored at 4°C. Meat Sci 2009, **81**, 689-692.