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Experimental evaluation of lysozyme as antimicrobial in minced meat

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Abstract

The present study examined the effect of lysozyme as a natural antimicrobial to prolong the shelf life of chilled minced meat. Lysozyme was added in two concentrations (100 and 200 mg/kg minced meat). Both treated and untreated minced meat samples were stored at 4˚C for ten days. Addition of lysozyme at 200 mg/kg reduced the aerobic plate count (APC), total psychrotrophic count and total yeast and mould count by 2.7, 2.8 and 2 log $_{10}$ CFU/g, respectively than the control samples in regard to bacterial count and also prolonged the shelf life of minced meat through retarding the deterioration of aroma and color. Lysozyme treated samples had lower total volatile basic nitrogen TVBN and Thiobarbituric acid TBA values than control samples without affecting pH of minced meat. The microbiology, chemical and sensory results indicated that treatment with lysozyme 200 mg/kg was the best sample among untreated and treated samples.

Keywords*: Lysozyme- minced meat- antimicrobial- sensory-TBA-TVBN*

Introduction

Meat has a wide range of nutrients and is a food preferred by many humans around the world as a source of animal protein. However, meat is conducive to the reproduction of many microorganisms, and as a result it spoils very easily. It is also sensitive to spoiling chemical and enzymatic activity. The decomposition of the fat, protein and carbohydrates contained in meat result in odors, flavors and appearance that is unpleasant, making it unsuitable for human consumption. Therefore, meat spoilage must be controlled in order to maintain nutritional value, texture and taste and to extend the shelf life **(Dave and Abdel 2011)**. Bacterial growth results in a deterioration of meat quality, as well as adverse changes in color and aroma **(Zhang *et al*., 2009).**

Raw minced meat constitutes a favorite environment for growth of pathogenic bacteria, causing severe food poisonings. Natural antimicrobials such as bacteriocins, essential oils, chitosan, or lysozyme may be alternatives for commonly used chemical preservatives **(Zhang *et al.,* 2009)**.

The important factor in the growth of microorganisms is the temperature of storing food at refrigeration to control the growth of psychotropic microorganisms, some pathogens and maintaining product quality. (**Pal. *et al*, 2008**).

Lysozyme is a constituent of hen egg white which has a long history of consumption worldwide, egg white is about 60% of the weight of an egg, where lysozyme is approximately 0.3%. Lysozyme was authorized as food additive in the EU with preservative antimicrobial effects (**FSAI, 2017)**.

Lysozyme consider a strong antibacterial activity against Gram-positive organisms. Due to increasing demand for natural food preservative, there is a practical application in the food processing industry. This enzyme is widely used as a preservative for meat, fish and their products. (**Radziejewska *et al*, 2008**).

Bacteriostatic and bacteriocidal properties of lysozyme have been used both to preserve various kinds of food as well as in pharmacy, human and veterinary medicine (**Rosiekandz and kolozyn – krajewska 2003)**.

Lysozyme is a type of enzymatic protein found in a wide range of organisms. Among the many applications of lysozyme, the antibacterial ac-

tivity features caused by the hydrolysis of 1–4 glycosidic bonds between N-acetylmuramic acid and N-acetylglucosamine of Gram positive bacteria are beneficial in the food industry, medicine, trade, and pharmacology **(Yang and Leśnierowski 2019).** Lysozyme, also known as muramidase or N-acetylmuramide glycanhydrolase, which is one of enzymes family (EC 3.2.1.17), that damage bacterial cell walls by catalyzing hydrolysis of 1, 4- beta- linkages between N-acetylmuramic acid and N- acetyl-D- glucosamine residues in a peptidoglycan and between N- acetyl-D- glucosamine residues in chitodextrins **(Sahoo *et al.,* 2012).**

Lysozyme can hydrolyze or dissolve bacterial cell wall specially, the cell wall of Gram-positive bacteria (that is, the peptidoglycan frame wrapping around the bacterial cells) which consider the main substrate for lysozyme, hence the susceptibility of this group of bacteria to lysozyme. **(Seo *et al.,* 2013).** In July 2018, the European Union (**EU**) approved the use of hen egg white lysozyme (HEWL) hydrolysates with a new food regulation **EU** 2018/991, allowing the use in food supplements for the adult population. Additionally, the Food and Drug Administration (FDA) recognized hen egg white lysozyme hydrolysate as generally recognized as safe (GRAS), allowing its use as an ingredient in supplements (**Vilcacundo *et al.,* 2018).** The aim of this study was to evaluate the effects of lysozyme on the microflora, color, aroma, TVBN, TBA and pH of minced meat.

Material and Methods

Lysozyme

Lysozyme (Biotech LDB0308, Canada) with the initial activity of 22,800 U/mg. Kewpie corporation (2017) suggested that usage of EWL is to add 50 ~ 200 ppm to the final product and it is convenient to use this as powder mix or water solution.

Minced Meat preparation

Minced meat obtained from retail market from Giza governorate, was divided into three samples of one kg each. The first sample constituted the control (without addition of lysozyme), the second was supplemented with lysozyme 100 mg per kilogram, the third sample was supplemented with 200 mg per kilogram lysozyme. All three portions were preserved at 4˚C for 10 days. During storage, microbiological analyses were performed, the color and odor of meat were evaluated, and pH, TVBN and TBA were measured. Analyses were conducted at zero, third, fifth, seventh, and tenth days.

Microbial analysis:

Preparation of minced meat homogenate perform according to ISO 6887-1/2017.

Enumeration of aerobic plate count and psychrotrophic bacteria were determined with plate count agar according to APHA 2001. APC were enumerated after incubation at 35^{o}C for 48h, while psychrotrophic bacteria enumerated after incubation at 7^{o}C for 10 days.

The number of yeasts and molds were determined with Dichloran Rose Bengal chloramphenicol agar enumerated after 5 day at 25^{o}C according to ISO 21527-1/2008.

Evaluation of aroma, color, and pH

Evaluation of changes in aroma was conducted by an experienced, trained nine-member sensory panel. The panel was composed of employees of the Animal Health Research Institute, Meat hygiene department, trained in basic methods of sensory analysis **(Polish Committee for Standardization 1996).** The same panelists were used throughout the entire study. Aroma was evaluated on a 5-point scale: a score of 5 was equivalent to very good aroma, and a score of 1 indicated poor aroma. The applied criteria in the evaluation of aroma of ground meat using a 5-point scale included the following: 5, intrinsic aroma, characteristic of fresh meat, desirable; 4, hardly detectable changes in aroma; 3, undesirable aroma, detectable changes, slightly altered; 2, distinctly altered aroma, of low intensity; 1, markedly altered, putrid, very intense aroma. The ground beef was also evaluated for worst point color and overall color. The panelists evaluated worst point color (1 = brown, 2 = moderately brownish red, 3 = slightly brownish red, 4 = dull red, 5 = bright red), which defines a discolored area of at least 2 cm in diameter, overall color (1 = brown, 2 = moderately brownish red, 3 = slightly brownish red, 4 = dull red, 5 = bright red) on days 0, 3, 5, 7 and 10 of display.

pH Measurement:

Meat sample (10 g) was homogenized with 50 mL deionized water for 1 min. pH was measured at room temperature using a digital pH meter (Suntex TS-1, Taiwan) equipped with a probe-type combined electrode (Ingold)

through direct immersion of electrode into the mixture **[EOS: 63-11/2006].**

Thiobarbituric acid (TBA)

According to **EOS**: **63-10/2006**

In a clean blender, about 10 gm of the examined sample were blended with 50 ml of D. W. for 2 minutes, and then were washed in distillation flask with 47.5 ml water. 2.5 ml of 4 M hydrochloric acid were added to bring the pH to 1.5, then was boiled till 50 ml distillate were obtained, then were filtrated. Five ml of TBA reagent (0.29 g/100 ml 90% glacial acid) were added to 5 ml of the filtrate in a screw capped test tube. The tubes were heating in a water bath for 35 minutes, and the absorbance of the resulting color was measured by using of a spectrophotometer (Spectronic21 Germany) at wave length 538 nm. The TBA values were recorded as mg malonaldehyde / Kg of the samples.

Concentration of malonaldehyde = 7.8 S mg/ Kg sample

Where S = the absorbance

Total volatile basic nitrogen (TVBN)

According to **EOS**: **63-9/2006**

Ten grams of the examined samples was added to 300 ml of distillated water and two grams of magnesium oxide then thoroughly mixed by a blender for 2 minutes and then was boiled till obtained 100 ml of distillate which received in flask contain 25 ml poric acid 2% and 2 drops of indicator. Flask was boiled tell 100 ml distillate was obtained. Sample was titrated with 0.1 M H_2SO_4 (R1). Steps were repeated using distilled water instead of sample as blank (R2).

TVBN mg/100 gm = (R1-R2) X 14.

Statistical analysis.

The experiment was repeated three times and the bacterial count were transformed to log CFU per gram. The results were subjected to statistical analyses using the ANOVA test by SPSS software package (IBM CO, version 20). The groups were separated from each other using LSD test.

Results and Discussion.

Lysozyme is an antimicrobial peptide that is effective against Gram-positive (and sometimes gram-negative) bacteria. It is naturally present in egg white, plants, and animal secretions. Its antimicrobial properties are associated with the hydrolysis of peptidoglycan layers in the bacterial cell wall and also with membrane perturbation **(Perez-Espitia *et al*., 2012)**. The total bacterial counts from all tested samples are presented in (**Table and chart 1**). For all treated and untreated samples, the lowest aerobic plate count (APC) was recorded in samples treated with 200 mg/kg lysozyme after 10 days of storage. The differences in APC between the control sample and the samples with an addition of 100 mg/kg and 200 mg/kg lysozyme were 2.3 and 2.7 $\log_{10}$ CFU/g, respectively throughout the whole storage period. There was significant difference between control sample and both treated samples from the beginning of storage period, while the significant difference between the two treatments appeared at the 7^{th} day of storage. Control samples exceeded the APC cited by **EOS 1694-2005** for minced meat 10^{6} CFU /g at the 7^{th} day of storage, while both treated samples did not exceed it. In a study by **Cannarsi *et al*. 2008**, it was found that total mesophilic viable counts and total psychrotrophic viable counts may be reduced only by immersion of meat steaks in a solution containing a combination of 0.5% lysozyme and 2% EDTA. **Radziejewska and Szablewski 2013** mentioned that the differences in APC between the control sample and the samples with an addition of modified lysozyme in heated and unheated meat were 1.5 and 1.1 log CFU/g, respectively. (**Table and chart 2)** showed the Psychrotrophes count ($\log_{10}$ cfu/g ± SD) of the examined samples during refrigerated storage. On the initial zero day the total psychrotrophic bacteria count in the control, 100 mg/kg and 200 mg/kg lysozymes samples were 3.9, 3.8 and 3.7 $\log_{10}$ CFU/g, respectively. While on day 10 of cold storage, the total psychrotrophic bacteria count of the meats treated with200 mg/kg lysozyme was found to be 3.1 $\log_{10}$ CFU/g with 2.8 $\log_{10}$ reduction from the control sample.

Fresh meat suffers during refrigerated storage some modifications such as physical, chemical and microbiological. The fresh meat is an excellent source of nutrients and is an ideal environment for the growth of spoilage microorganisms and common pathogens, the presence of aerobic conditions leads to growth of mainly aerobic psychrotrophic bacteria types which responsible for fresh meat spoilage during aerobic cold storage. **(D. Djenane and P. Ron-**

cales 2018).
The microbial spoilage of meat occurs when counts of aerobic bacteria reach levels to 7 $\log_{10}$ CFU/g (**Djenane D *et al* 2016**).
Ercolini D. *et al*. (2009) identified mesophilic and psychotropic populations from refrigerated meat, from eight samples stored for 10 days under vacuum showed viable counts determined on PCA ranging from 7.0×10^5 to 5.8×10^6 cfu/g for mesophilic bacteria and from 1.1×10^6 to 2.5×10^7 cfu/g for Psychrotrophic bacteria.
Temperature is the most important factor for controlling the food quality and safety because of its effect on microbial growth, although the low temperature can reduce the growth rate of many species of microorganisms but psychotropic microorganisms can grow at normal refrigeration temperatures **(Marklinder *et al*., 2004).**
Results illustrated in **(Table and chart 3)** showed the total yeast and mold count ($\log_{10}$ cfu/g ± SD) of the examined samples during refrigerated storage. **Stagnitta, *et al*. (2006)** enumerate the counts of molds and yeasts in 515 meat samples from retail stores in San Luis City were 10^3 and10^6 cfu/g, Argentina Alimentary Code (AAC) has no regulation for these microorganisms, similar to that found in **EOS** 1694-2005 where there is no limit for molds and yeasts. **Benkerroum (2008**) reported that lysozyme has inhibitory activity on fungi despite the absence of typical peptidoglycan in their envelopes. These inhibition of yeast and mold was due to presence of chitin as an important constituent of their cell wall so lysozyme possesses a chitinase activity.
The mean count of total yeast and mold were 4.3±0.1, 2.6±0.1 and 2.3±0.1 at the end of storage period in the control, 100 mg/kg and 200 mg/kg lysozyme treated samples. The highest reduction was recorded in samples treated with200 mg/kg lysozyme. **Sawasdipuksa *et al*., 2011** found that lysozyme expressed antifungal activity with a rather high thermal stability of up to 80 °C for 15 min (at pH=8.0). It exerted an antifungal action towards *Macrophomina phaseolina* but displayed no antifungal activity against two other isolates, *Phymatotrichopsis omnivora* and *Fusarium avenaceum*.
The initial pH range of untreated minced meat and meat treated with both lysozyme concentrations were 5.72 to 5.81. The addition of lysozyme had no significant effect on pH. These results were similar to that obtained by **Radziejewska and Szablewski 2013,** who stated that the addition of modified lysozyme had no significant effect on pH. **Insausti *et al.*, 2001** found that the mean values of pH of fresh beef around 5.56± 0.09. The increase of pH may be due to the partial proteolysis leading to the increase of free alkaline. Ammonia is one of the most spoilage end products in spoiled meat which is directly responsible for spoilage odors and flavors, it is considered as an indicator for amino acid degradation by bacteria and it can be measured as total volatile basic nitrogen **(Edris *et al.*, 2013).** Meat fit for human consumption, their TVB-N contents should not exceed 20mg /100g stipulated by **(EOS. 1694/2005).** From the results reported in **(table 5)** and **(Chart 5)** it is clear that the TVB-N values exceeded the critical limit at the 10^{th} day of storage in the control sample (21.8), while both treated samples were below the critical limit even at the end of storage period.
Malondialdehyde (MDA) is a degradation product of lipid oxidation not only influences the eating quality, but also has harmful effects on the human health, this product criticized as a carcinogenic factor in food. **(Djenane D. and Roncales. P. 2018).**
According to **(EOS. 1694/2005).** which recommended that TBA of fresh meat should not exceed 0.9 malondialdehyde /Kg, TBA value of the control samples exceeded the critical limit from the 7^{th} day of storage, while TBA values of both treated samples were below the critical limit tell the end of the storage period. It is of great importance to mention that TBA values could be a useful quality index for the assessment of rancidity during the storage of lipid rich **(Wilson 1991).**
Sanchéz *et al*. (2001) stated that oxidation rate of lipid depends on the presence of O_2, the prooxidants/antioxidants balance, the degree of unsaturated fatty acids and the storage conditions to control all these factors to retard lipid oxidation, off- flavor and off - odors by using antioxidants and chelating agent as inhibitors of lipid oxidation.
Korzycka and Jarmoluk (2015) stated that

lysozyme significantly increases antioxidant activity in meat. **Liu *et al*., 2006** and **Mine *et al.*, 2004** reported that lysozyme has a strong antioxidant property as it scavenges free radicals and hydroxyl molecules, leading to decreased oxidative stresses. A considerable deterioration of aroma and color of samples in the case of control one was observed at the 7^{th} day of storage, while the 100 mg/kg lysozyme samples deteriorated at the 10^{th} day of storage. However, the 200 mg/kg lysozyme samples were still having high score for both color and aroma tell the end of storage period. Similar results concerning the applicability of lysozyme in the extension of shelf-life of meat and meat products were obtained by other authors **(Gill and Holley 2000; Malicki *et al*., 2004 and Nattress *et al*., 2001)**. Fresh beef shelf-life based on sensory analysis, chemical, physical and microbiological properties. Thus **Djenane D. *et al*. (2016)** defined it as the period between slaughter of animal till retail purchasing during the meat retains all its qualities attributes.

The results indicate that lysozyme might be an effective agent extending shelf-life of minced meat.

Conclusions and Recommendations:

The microbiology, chemical and sensory results obtained by this article indicated that treatment with lysozyme 200 mg/kg was the best sample among untreated and treated samples. Lysozyme significantly reduced the APC, psychrotrophic and total yeast and mould counts, as well as hindering the deterioration of meat stored at recommended, temperature ($\leq 4^{O}C$). Finally, we concluded that lysozyme could be used as an effective agent extending shelf-life of minced meat.

Table (1). Aerobic plate count (APC) (log_{10} cfu/g ± SD) of the examined samples during storage at $4^{o}C$

	Zero	**3**	**5**	**7**	**10**
C	5.1^{A} ±0.1	5.4^{A} ± .1	5.9^{A} ±0.1	6.4^{A} ±0.1	6.9^{A} ±0.1
Lz100	4.9^{a} ±0.1	5^{a} ±.1	5^{aB} ±0.1	4.8^{aB} ±.1	4.6^{aB} ± 0.1
Lz200	4.8^{a} ±0.1	4.9^{a} ±0.1	4.7^{ab} ±0.1	4.5^{aB} ±0.1	4.2^{ab} ±0.1

C: Control **SD**: Standard deviation **Lz:** lysozyme
There are sig. diff. (P<0.05) between means having the same capital and small letters in the same column.
*: results shown are means of triplicates of each group**.**

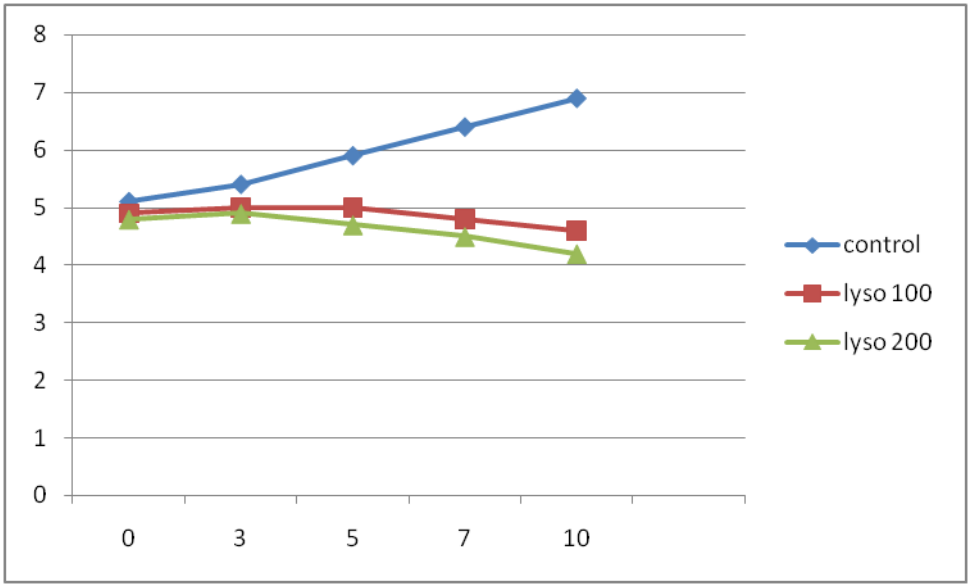

Chart (1). Total bacterial count of the examined samples during storage at 4°C.

Table (2). Psychrotrophes count (log_{10} cfu/g ± SD) of the examined samples during storage at 4°C

	Zero	3	5	7	10
C	3.9 ^{A} ±0.1	4.5 ^{A} ± .1	4.9 ^{A} ±0.1	5.2 ^{A} ±0.1	5.9 ^{A} ±0.1
Lz100	3.8 ^{a} ±0.1	4 ^{a} ±.1	4.1 ^{aB} ±0.1	3.8 ^{aB} ±.1	3.5 ^{aB} ±0.1
Lz200	3.7 ^{a} ±0.1	3.9 ^{a} ±0.1	3.7 ^{ab} ±0.1	3.5 ^{ab} ±0.1	3.1 ^{ab} ±0.1

C: Control SD: Standard deviation Lz:lysozyme
There are sig. diff. (P<0.05) between means having the same capital and small letters in the same column.
*: results shown are means of triplicates of each group.

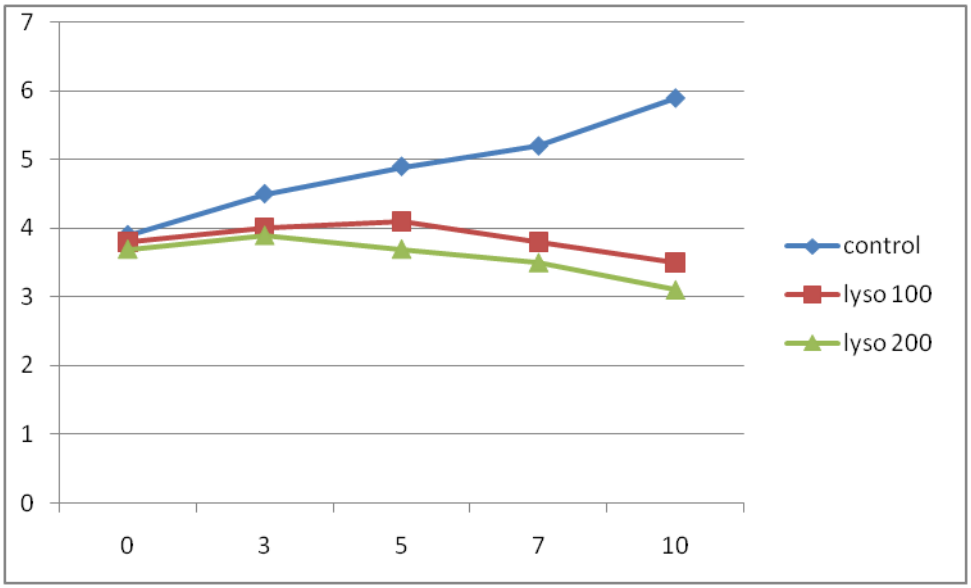

Chart (2). Psychrotrophes count of the examined samples during storage at 4°C

Table (3). Yeast and mold count (log_{10} cfu/g ± SD) of the examined samples during storage at 4°C

	Zero day	3rd day	5th day	7th day	10th day
C	3.2^{A} ±0.1	3.5^{A} ± 0.1	3.6^{A} ±0.1	4.2^{A} ±0.1	4.3^{A} ±0.1
Lz100	3^{a} ±0.1	3.2^{a} ±0.1	3^{aB} ±0.1	2.8^{aB} ±.1	2.6^{aB} ±0.1
Lz200	3^{a} ±0.1	3.1^{a} ±0.1	2.8^{ab} ±0.1	2.6^{ab} ±0.1	2.3^{ab} ±0.1

C: Control **SD**: Standard deviation **Lz:**lysozyme
There are sig. diff. (P<0.05) between means having the same capital and small letters in the same column.
*: results shown are means of triplicates of each group**.**

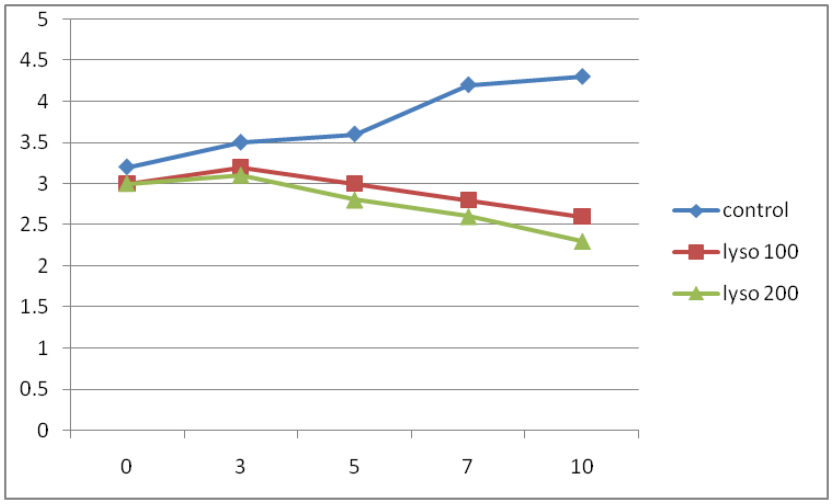

Chart (3). Yeast and mould count of the examined samples during storage at 4°C.

Table (4). Mean value of TBA of treated samples (mean of 3 trials ± SD) during storage at 4°C.

	Zero day	3rd day	5th day	7th day	10th day
C	$0.5^{a\,I}$ ±0.12	$0.7^{a\,b\,I}$±0.07	$0.8^{b\,c\,I}$ ±0.1	$1.0^{c\,I}$ ±0.08	$1.2^{d\,I}$ ±0.05
Lz100	$0.5^{a\,I}$ ±0.12	$0.6^{a\,b\,I}$±0.08	$0.7^{b\,I}$ ±0.08	$0.8^{b\,I\,II}$±0.08	$0.9^{c\,II}$ ±0.08
Lz200	$0.5^{a\,I}$ ±0.12	$0.6^{a\,b\,I}$±0.08	$0.7^{b\,I}$ ±0.1	$0.7^{b\,c\,II}$ ±0.1	$0.9^{c\,II}$ ±0.05

*No significant difference (P<0.05) between cells contain same alphabetical letter in the same raw.
*No significant difference (P<0.05) between cells contain same latin letter in the same column.

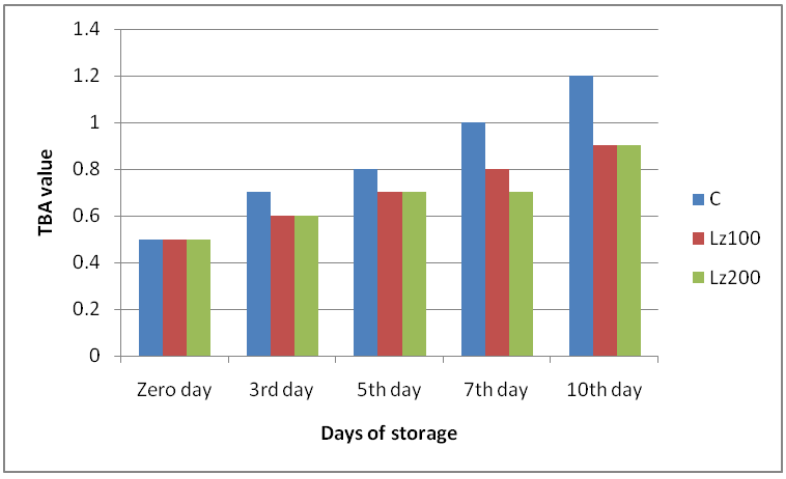

Chart (4). Mean value of TBA of treated samples.

Table (5). Mean value of TVB-N of treated samples (mean of 3 trials ± SD) during storage at 4°C.

	Zero day	3rd day	5th day	7th day	10th day
C	$13.5^{a\,I} \pm 0.86$	$14.7^{a\,I} \pm 0.69$	$15.7^{a\,b\,I} \pm 1.7$	$18.2^{b\,I} \pm 1.6$	$21.8^{c\,I} \pm 1.9$
Lz100	$12.6^{a\,I} \pm 1.4$	$13.4^{abI} \pm 1.03$	$14.2^{a\,d\,I} \pm 1.4$	$15.5^{dcII} \pm 0.8$	$17.7^{c\,II} \pm 1.7$
Lz200	$11.3^{a\,I} \pm 0.86$	$12.4^{abII} \pm 0.7$	$13.5^{b\,c\,I} \pm 1.2$	$14.8^{c\,II} \pm 0.7$	$17.0^{d\,II} \pm 1.7$

*No significant difference (P<0.05) between cells contain same alphabetical letter in the same raw.
*No significant difference (P<0.05) between cells contain same latin letter in the same column.

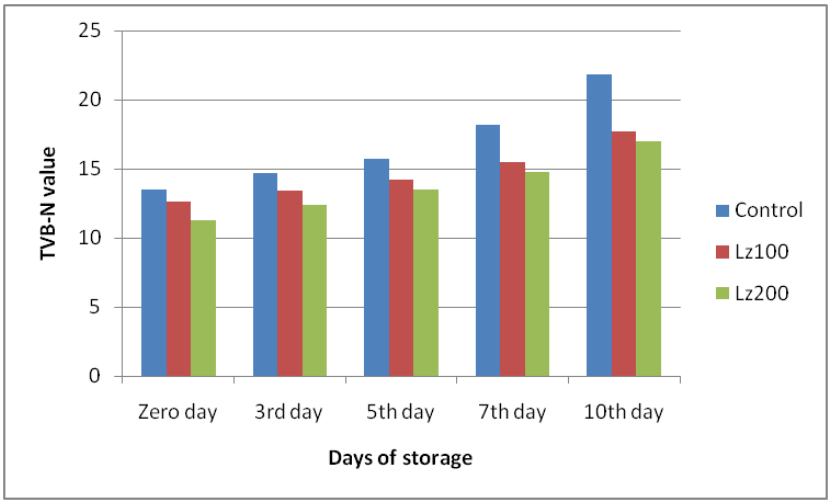

Chart (5). Mean value of TVB-N of treated samples.

Table (6). Mean value of pH value of treated samples (mean of 3 trials ± SD) during storage at 4°C.

	Zero day	3rd day	5th day	7th day	10th day
C	$5.5^{a\,I} \pm 0.17$	$5.6^{a\,b\,I} \pm 0.14$	$5.6^{a\,c\,I} \pm 0.12$	$5.7^{a\,d\,I} \pm 0.08$	$5.7^{bcd\,I} \pm 0.05$
Lz100	$5.5^{a\,I} \pm 0.14$	$5.5^{a\,I} \pm 0.1$	$5.6^{a\,b\,I} \pm 0.1$	$5.6^{a\,c\,I} \pm 0.07$	$5.7^{bc\,I} \pm 0.02$
Lz200	$5.5^{a\,I} \pm 0.14$	$5.5^{a\,I} \pm 0.12$	$5.6^{a\,I} \pm 0.08$	$5.6^{a\,I} \pm 0.08$	$5.6^{a\,I} \pm 0.07$

*No significant difference (P<0.05) between cells contain same alphabetical letter in the same raw.
*No significant difference (P<0.05) between cells contain same latin letter in the same column.

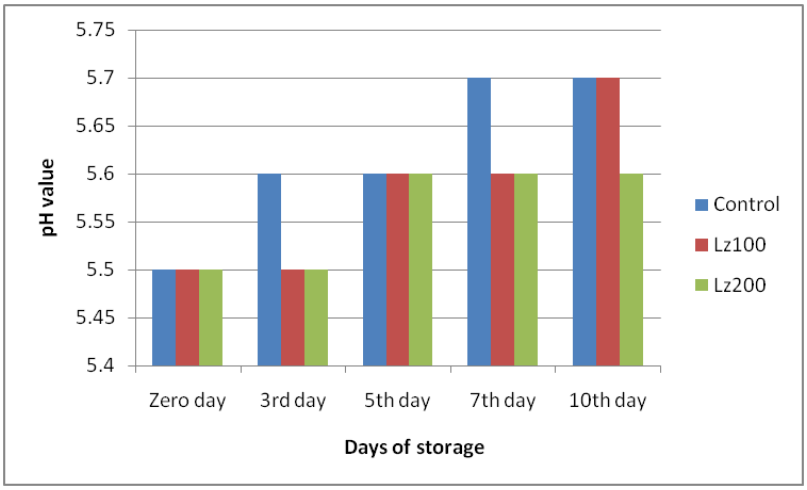

Chart (6). Mean value of pH of treated samples.

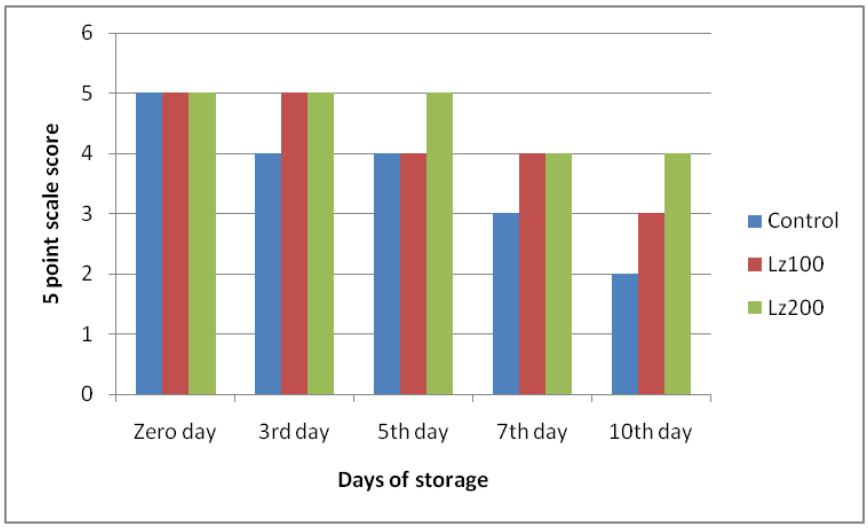

Chart (7). Effects of lysozyme on aroma and color of ground beef during refrigerated storage.

References

(FSAI) (2017). Food safety authority of Ireland safety assessment of lumivida (Hen egg white lysozyme hydrolysate). DSM nutritional Products LTD.

APHA: **American public health association (2001).** Compendium of methods for microbiological examination of food.

Benkerroum, N. (2008). Antimicrobial activity of lysozyme with special relevance to milk. African J. Biotech. 7 (25); 4856 : 4867.

Cannarsi, M.; Baiano, M.; Sinigaglia, L.; Ferrara, R.; Baculo, A. and Del Nobile, M. A. (2008). Use of nisin, lysozyme and EDTA for inhibiting microbial growth in chilled buffalo meat. Int. J. Food Sci. Technol. 43: 573–578.

Dave, D. and Abdel, E.G. (2011). Meat spoilage mechanisms and preservation techniques: A critical review. Am. J. Agri. & Biol. Sci. 6: 486-510.

Djenane, D. and Roncales; P. (2018). Carbon monoxide in meat and fish packaging advardages and limits. Foods (MDPI).,7; 1: 34.

Djenane, D.J. Beltran, J.A.; Camo, J. and Roncalés, P. (2016). Influence of vacuum at different ageing times and subsequent retail display on shelf- life of beef cuts package with active film under high O_2. J. food Sci. Technd. 53; 4244-4257.

Edris, A.; Hassan, M.; Saltout, F. and El-Hosseny, S. (2013). Chemical evaluation of cattle and camel meat. BENHA VETERINARY MEDICAL JOURNAL, 25(2):145-150.

Egyptian standard (EOS) (2005). Egyptian Organization for specialization and quality control, Egyptian Standard for fresh meat. Ministry of Industry No. 1522/2005.

EOS 1694-(2005). Egyptian standards for frozen minced meat.

EOS: 63-10/(2006). Methods of analysis and testing for meat and meat products part 10. Determination of Thiobarbituric acid (TBA).

EOS: 63-11/(2006). Methods of analysis and testing for meat and meat products part 11. Measurement of pH.

EOS: 63-9/(2006). Methods of analysis and testing for meat and meat products part 9. Determination of Total volatile nitrogen.

Ercolini, D.; **Russo, F.; Nasi, A.; Ferranti, P. and Villain, F. (2009).** Mespohilic and Psychotrophic bacteria from meat and their

spoilage potential in vitro and in beef. Appl. Environ. Microbiol. 75 (7): 1990-2001.

Gill, A.O. and Holley R.A. (2000). Inhibition of bacterial growth on ham and bologna by lysozyme, nisin and EDTA. Food Res Int. **33**, 83-91.

Insausti, K.; Beriain, M.J.; Purroy, A. Albirti, P. and Alzueta, M. (2001). Shelf life of beef from local Spanish cattle breeds stored under modified atmosphere. Meat Sci., 57(3): 273-281.

ISO 6887-1/ (2017). Microbiology of food and animal feeding stuffs -- Preparation of test samples, initial suspension and decimal dilutions for microbiological examination -- Part 2: Specific rules for the preparation of meat and meat products.

ISO 21527-1/(2008). Microbiology of food and animal feedings stuffs — Horizontal method for the enumeration of yeasts and moulds — Part 2: Colony count technique in products with water activity less than or equal to 0,95

Kewpie corporation fine chemical Divison Japan (2017). food additive Egg white lysozyme. http//www.kewpie.co.JP

Korzycka, A.Z. and Jarmoluk, A. (2015). The use of chitosan, lysozyme, and the nanosilver as antimicrobial ingredients of edible protective hydrosols applied into the surface of meat. J Food Sci Technol. 52(9): 5996–6002.

Liu, H.; Zheng, F.; Cao, Q.; Ren, B.; Zhu, L.; Striker, G. and Vlassara, H. (2006). Amelioration of oxidant stress by the defensin lysozyme. *American Journal of Physiology, Endocrinology Metabolism*; 290: E824.

Malicki, A.; Jarmoluk, A. and Brużewicz, S. (2004). 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, 48, 47-51.

Marklinder, I.M.; Lindbland, M.; Eriksson, L.M; Finnson, A.M. and Lindquist, R. (2004). Home storage temperatures and consumer hand ling of refrigerated foods in Sweden. J. food Prot., 67; 2570 : 2577.

Mine, Y.; Ma, f. and Lauriau, S. (2004). Antimicrobial Peptides Released by Enzymatic Hydrolysis of Hen Egg White Lysozyme, Journal of Agricultural and Food Chemistry;52**,** 1088-1094.

Nattress, F.M.; Yost, C.K. and Baker, L.P. (2001). Evaluation of the ability of lysozyme and nisin to control meat spoilage bacteria. Int J Food Microbiol, 70, 111-119.

Perez-Espitia, P.J.; Ferreira-Soares, N.F.; Coimbra, J.S.R.; Andrade, N.J.; Cruz, R.S. and Alves-Medeiros, E.A. (2012). Bioactive peptides: synthesis, properties, and applications in the packaging and preservation of food. Comp. Rev. Food Sci. Technol. 11: 187-204.

Pol, A.; **labuza, T.P. and Diez- Gonzalez, F. (2008).** Ready to eat sliced uncured turkey breast and cared hom under probable storage conditions based on listeria moncytogenes and psychrotroph growth. Int. J. food Microbiol., 126, 48 : 56.

Polish Committee for Standardization (PKN). (1996). PN-ISO 8586- 1:1996. Sensory analysis—general guidance for the selection, training and monitoring of assessors. Polish Committee for Standardization, Warsaw. Available at: http://www.pkn.pl/en.

Radziejewska, R.C. and Szablewski, T. (2013). Effect of Modified Lysozyme on the Microflora and Sensory Attributes of Ground Pork. Journal of Food Protection, 76, (2): 338 –342.

Radziejewska, R.C.; Lesnierowski, G. and Kijowski, J. (2008). properties and application of egg white lysozyme and its matified preparations – Areview polish J. of food and Nutrition sciences, 58 (1): 5-10.

Rosiak, E. and Kolozyn – Krajewska d.

(2003). Application of prognostic microbiology methods for evaluation of growth of saprophytic bacteria in meat products presorted with lysozyme in monomer form. Zywnosé, 36 (3): 5-20.

Sahoo, N.R.; Bhusan, B.; Bhattacharya, T.K.; Dayal, S. and Sahoo, M. (2012). Lysozyme in livestock: A guide to selection for disease resistance. A Reeview J. Anim. Sci. Adv., 2: 347-360.

Sanchéz, A.; djenane, D.; Torrescano, G., Beltran, J.A. and Roneales, P. (2001). The effects of ascorbic acid, to urine, carnosine and rosemary powder on color and lipid stability of beef patties packaged in modified atmosphere. Meat so. 58, 421: 429.

Sawasdipuksa, N.; Lei, Z.; Sumner, L. W.; Niyomploy, P. and Sangvanich, P. (2011). A Lysozyme with Antifungal Activity from Pithecellobium dulce Seeds. Food Technol. Biotechnol. 49 (4): 489–494.

Seo, S.; Karboune, S.; L'Hocine, L. and Yaylayan, V.A. (2013). Characterization of glycated lysozyme with galactose, galactooligosaccharides and galactan: effect of glycation on functional properties of lysozyme. LWT: Food Sci. Technol 53: 44–53.

Stagnitta, P.V.; Micalizzi, B. and Stefanini de Guzmán, A.M. (2006). Prevalence of some bacteria yeast, and molds in meat foods in San luis, Argentina, cent. EUR, J. publ. health, 14 (3); 141-144.

Vilcacundo, R.; Méndez, P.; Reyes, W.; Romero, H.; Pinto, A. and Carrillo, W. (2018). Antibacterial Activity of Hen Egg White Lysozyme Denatured by Thermal and Chemical Treatments. Sci. Pharm. 86, 48:4-17.

Wilson, A. (1991). Practical meat inspection, Blackwell, Scientific Publication, Oxford. U.K. 5th Ed.

Yang, T. and Leśnierowski, G. (2019). Changes in selected physicochemical properties of lysozyme modified with a new method using microwave field and oxidation. PLoS ONE 14(2):1-9: e0213021. https://doi.org/10.1371/journal.pone.0213021

Zhang, H.; Kong, B.; Xiong, Y.L. and Sun, X. (2009). Antimicrobial activities of spice extracts against pathogen and spoilage bacteria in modified atmosphere packaged fresh pork and vacuum packaged ham slices stored at 4uC. Meat Sci. 81:689–692.