

HIGHLIGHT ON THE INFLUENCE OF LACTIC ACID AND OZONIZED WATER ON THE SHELF LIFE OF CHICKEN FILLET

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ABSTRACT

The study was supposed to detect the impact of three decontaminators (ozonized water, lactic acid and their blend of them) in improving organoleptic characteristics, chemical and bacteriological quality of poultry meat. Chicken fillet samples were collected from Zagazig city, Egypt. and divided into three groups, Group1: To assess the impact of additives on sensory criteria, chemical quality indices (pH, Thiobarbituric acid, Total Volatile Basic-Nitrogen) and APC of the examined samples with reference to their shelf life, Group 2: For assessment the additives effectiveness on *Enterobacteriaceae* counts, Group 3: to evaluate the influence of additives on *Staphylococcus aureus* artificially inoculated in chicken fillet meat samples, each group was divided into 4 subgroups (control, ozonized water 0.38%, lactic acid 1% and blend of both), the data cleared that the immersion of samples in the blend of ozonized water with lactic acid can prolong the shelf life of chicken fillet during chilled storage at 4 °C ±1 with keeping its sensory characteristics and chemical parameters meanwhile a combination of ozonized water and lactic acid can protract the shelf life of chicken fillet by additional 48 hours. Concerning to the antimicrobial efficacy of the different trials, it's interesting to note that the combined uses of ozonized water –lactic acid have greatly a noticeable decrease in aerobic plate count, *Enterobacteriaceae* count and *S. aureus* count, followed by ozonized water, finally lactic acid. So it's recommended that using ozonized water-lactic acid blend in poultry meat.

Keywords: Ozonized water, lactic acid, *Enterobacteriaceae*, *S.aureus*.

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INTRODUCTION

An outstanding nutritional profile is a characteristic of chicken meat as it is vital to include in a diet plan for people of all ages because it is low in fat and nearly entirely composed of unsaturated fatty acids, as well as high biological value protein, vitamins, and minerals (Marangoni *et al.*, 2015) but, as soon as the fresh meat processing process starts, contamination occurs. Usually, the animal is the source of pollution or its external surroundings, as contamination occurs first on the external surface of the meat, and because of the extended production, packing, and transportation chain, meat provides the excellent medium for microbial growth (Capita *et al.*, 2001). The most dangerous contaminant is the bacteria such as *Staphylococcus* that can be present in most meals for example red meat, chicken and their products which are directly manipulated by humans (Kitai *et al.*, 2005). In addition of producing virulent heat-stable toxins that cause toxic shock syndrome, *S. aureus* is a particularly dangerous microorganism that can cause hospital infections and food poisoning (Kérouanton *et al.*, 2007). *S. aureus* is the most familiar reason of food-borne human sickness worldwide (Do Carmo *et al.*, 2004). *Enterobacteriaceae* are of the most bacteria isolated from chicken meat. The *Enterobacteriaceae* family is sub grouped into 8 tribes including: *Escherichie* spp, *Edwardsiella* spp, *Salmonelle* spp, *Citrobactere* spp, *Klebsielle* spp, *Protee* spp, *Yersine* spp, and *Erwine* spp (Ardrey *et al.*, 1968)

Consequently, many consumers have expressed a desire for higher-quality chicken products with a high dietetic value, harmless food additives, prolonged shelf lives, and most importantly, no pathogenic microorganisms. (Sarron *et al.*, 2021).

One of the food improvement techniques able to deactivate bacteria is by ozonation method. Odor control, color removal,

organic compound decomposition, and air and water disinfection have all been achieved with ozone. Ozonized water is very hazardous to bacteria due to its great oxidation potential (Qingshi *et al.*, 1989). Furthermore, ozonized water had no adverse effects on the organoleptic criteria of chicken products (Mancini and Hunt, 2005). Ozonized water was accepted by the USDA as an appropriate and safe component for use in preparation of meat and poultry (United States Department of Agriculture, 2002).

Lactic acid is an organic acid with confirmed efficiency as a decontaminant in many categories of food. It can prolong the shelf life of chicken while it is refrigerated by slowing the growth of bacteria that cause spoiling, preventing the production of unwanted compounds, and enhancing sensory qualities (Smaoui *et al.*, 2012). The maximum initial reduction in aerobic mesophilic and psychophilic bacteria in chicken breast was achieved by lactic acid 3% (Cosansu *et al.*, 2011).

Between the effective treatment practices is the mixing of organic acid solutions and another antibacterial agent that have been tested on the chicken products and showed variable results. Additions of LA to ozonized water enhance its decontamination power (Megahed *et al.*, 2020). So, the current study was aimed to explore the effect of ozonized water 0.38%, lactic acid 1% and their blend on sensory characteristics, biochemical profile, shelf life and microbial load of chicken fillet as a raw material of most chicken products.

MATERIALS AND METHODS:

1- Preparation of chicken fillet samples:

Chicken fillets were collected from the city market in Zagazig, handled under aseptic conditions and transported an ice box container without delay to microbiology laboratory at Animal Health Research Institute, Zagazig Lab., for more examination.

Aerobic Plate Count, *Enterobacteriaceae* and *S. aureus* count were detected according to APHA (2001) and ISO 21528-2, 2004 and FDA (2001) in a preliminary, unreported trial to obtain the samples of choice for further treatment trials with different decontaminators, i.e. samples with a known total bacterial and *enterobacteriaceae* count and *S.aureus* free samples.

2- Preparation of decontaminators:

2.1. Lactic acid: Lactic acid prepared in a concentration of 1%

2.2. Ozonized water: Ozonized water was prepared by ozonating tap water with an ozone generator and used immediately after the wanted concentration was obtained (0.38). (Karamah and Wajdi, 2018)

2.3. Ozonized water 0.38- lactic acid 1% blend (1:1)

3- Preparation of *S. aureus* culture suspension:

Staphylococcus aureus ATCCC6538™ obtained from Animal Health Research Institute were refreshed on Baird Parker media with Egg yolk-Tellurite emulsion, incubated at 37°C for 24h, the colonies were picked up and inoculated into Brain Heart Infusion broth until the turbidity was adjusted to match a 0.5 McFarland standard tube (1.5×10^8 CFU) and incubated at 37°C for 24h, Then centrifugation for 15 minutes at 3000 rpm to obtain a pellet of bacterial cells, then washing twice in phosphate buffered saline (PBS) and diluted to 1.0×10^6 CFU/ ml in PBS for inoculating of the samples Saad *et al.* (2015).

Design of the experiment:

Three groups of chicken fillet samples were subjected to the following treatment

Group 1: For evaluation of the effectiveness of additives on sensory criteria, chemical quality indices (pH, TBA and TVB-N) and APC of the examined samples, the analysis

were done initially after 1 hr. (1st day) then day after day until decomposition take occur.

Group 2: For evaluation of the effectiveness of additives on *Enterobacteriaceae* count in treated and control samples

Group 3: For evaluation of the effectiveness on *S. aureus* count artificially inoculated in chicken fillet samples after 30, 60 and 120 minutes of dipping in decontaminators.

Each group was subdivided into four sub-groups (control, lactic acid 1%, ozonized water 0.38% and ozonized water 0.38- lactic acid 1% blend (1:1), 3 trials for each).

The control and treated groups were chilled at 4 °C \pm 1 with interval period (1-9 days) were subjected to further sensory, biochemical and bacteriological analysis

4-Sensory evaluation:

The coded samples were organoleptically examined in the period of refrigerated storage for color, odor, texture and overall acceptability by panel consisting of 10 members from the Animal Health Research Institute. The panelists used a 9-rating scale for their scores as described by Pearson and Tauber (1984).

5- Chemical quality parameters:

5.1- pH (Pearson, 2006):

Approximately, 10 grams of the sample and 10 ml of neutralized distilled water were mixed in a blender. After 10 minutes of continuous shaking at room temperature, the homogenate was allowed to settle. Then use an electrical pH meter and record results.

5.2- Determination of Total Volatile Basic Nitrogen (TVB-N):

According to Food and Agriculture Organization "FAO"(1980).

5.3- Determination of Thiobarbituric Acid Number (TBA):

According to Pikul *et al.* (1989).

6- Bacteriological evaluation:

Preparation of samples: ISO 6887-3 (2017):

From control and treated groups, 10 g test portion was taken under aseptically, transferred into a sterile 90 ml peptone water 0.1% (Merck) to obtain a dilution 10^{-1} , a millimeter from this suspension (10^{-1}) was transferred with a sterile pipette to a tube of sterile peptone water 0.1 % (9 ml) to obtain a dilution of (10^{-2}), repeat this step for more serial dilutions.

Enumeration and isolation procedure:

1- Aerobic plate count (APC): APHA (2001):

Aseptically, 0.1 ml of each dilution was spread onto the surfaces of two sets of plate count agar solid media in petri dishes that had already been labeled. After letting the medium absorb the inoculum, invert the plates and incubate them for 48 hours at 35 ± 2 °C. The duplicate plate average count was counted, and the APC/g was calculated.

2- Enumeration of *S. aureus* (BAM, FDA, Chapter 12):

Pouring of 1 ml of the prepared dilution into plates containing the specific media (Baird Parker - Egg yolk-Tellurite emulsion) incubated at 35 -37 °C for 48 hours. To detect the count of *S. aureus* per gram,

distinctive black colonies with a zone of clearing and a narrow white margin surrounding them were counted.

3-Enumeration of Enterobacteriaceae (ISO 21528-2, 2004)

Using poured-plate technique 1 ml of the initial suspension is inoculated in two Petri dishes then pouring of Violet red bile glucose agar (44 °C to 47 °C). After solidification a layer of the same medium is added and allows solidifying. The dishes are incubated at 37 °C for 24 h \pm 2 h. Confirmation Subculture of colonies of presumptive *Enterobacteriaceae* on non-selective medium, and confirmation by means of tests for fermentation of glucose and presence of oxidase.

7- Statistical analysis

The recorded results were statistically analyzed using analysis of variance (ANOVA) test and comparative of means were performed according to Duncan, using SPSS ver. 14 (2006). All bacterial counts were changed to \log_{10} (log CFU/g). Results were recorded as mean \pm standard errors (SE). The value of $P < 0.05$ was used to indicate statistical significance.

RESULTS

Table 1: Sensory evaluation of treated and untreated chicken fillet samples.

Keeping quality	Fit for human utilization	Border line	Decomposed
Control samples (Untreated)	0-5 th day	5 th - 6 th day	7 th day
Samples treated ozonized water	0-6 th day	6 th - 7 th day	8 th day
Samples treated with lactic acid	0-9 th day	9 th - 10 th day	11 th day
Treated samples with ozonized water - lactic acid blend	0 - 9 th day	9 - 10 th day	11 th day

Table 2: The mean pH values in examined treated and untreated chicken fillet samples.

Treated groups Durations	Control	ozonized water	Lactic acid 1%	Ozonized water - lactic acid blend
	1 st day	5.58± 0.03 ^a	5.87 ± 0.04 ^a	5.65 ±0.02 ^b
3 rd day	6.13± 0.029 ^a	5.90± 0.01 ^b	5.90 ±0.01 ^b	5.91 ±0.04 ^b
5 th day	6.61 ±0.024 ^a	6.18 ± 0.015 ^b	6.02±0.001 ^c	6.14 ±0.014 ^{bc}
7 th day	-	6.59 ±0.07	6.18 ±0.02	6.80 ±0.06
9 th day	-	-	6.77 ±0.014	7.08 ±0.03

Means inside the same column with different superscripts are different significantly at ($p < 0.05$) according to Duncan's multiple comparisons.

Table 3: The mean TVB-N values in examined treated and untreated chicken fillet samples.

Treated groups Durations	Control	ozonized water	Lactic acid 1%	Ozonized water - lactic acid blend
	1 st day	6.56± 0.17	6.84 ± 0.017	6.39 ±0.009
3 rd day	12.85± 0.23 ^a	8.85± 0.19 ^b	7.85 ±0.09 ^c	7.80 ±0.1 ^c
5 th day	21.30 ±0.36 ^a	14.91 ±0.62 ^b	14.10 ±0.23 ^b	13.69 ± 0.43 ^b
7 th day	-	22.26 ±0.49	17.14 ±0.08	19.17 ±0.17
9 th day	-	-	20.41 ±0.33	21.63 ±0.31

Means inside the same column with different superscripts are different significantly at ($p < 0.05$) according to Duncan's multiple comparisons.

Table 4: The mean TBA values in examined treated and untreated chicken fillet samples.

Treated groups Durations	Control	ozonized water	Lactic acid 1%	Ozonized water - lactic acid blend
	1 st day	0.087± 0.01	0.09 ±0.01	0.074 ±0.003
3 rd day	0.55±0.03 ^a	0.48± 0.03 ^b	0.35 ±0.01 ^c	0.33±0.02 ^c
5 th day	1.03 ±0.05 ^a	0.82 ±0.01 ^b	0.67±0.01 ^c	0.62±0.05 ^c
7 th day	-	1.19 ±0.12	0.91 ±0.01	0.90±0.02
9 th day	-	-	1.20 ±0.08	1.13±0.06

Means inside the same column with different superscripts are different significantly at ($p < 0.05$) according to Duncan's multiple comparisons.

Table 5: The mean APC±S.E (Log₁₀ CFU/g) of untreated and treated groups of chicken fillet samples.

Treated groups Durations	Treated groups			
	Control	ozonized water	Lactic acid 1%	Ozonized water - lactic acid blend
1 st day	5.7± 0.9 ^a	4.22 ± 0.15 ^b	4.12 ±0.11 ^{bc}	3.83 ±0.07 ^c
3 rd day	6.8± 0.11 ^a	2.9± 0.18 ^c	3.50 ±0.10 ^b	2.40 ±0.04 ^d
5 th day	7.7± 0.05 ^a	4.93 ± 0.07 ^b	4.33±0.11 ^c	3.24 ±0.11 ^d
7 th day	R	6.02 ± 0.10	5.56 ± 0.27	4.92 ±0.25
9 th day	R	R	7.02 ± 0.05	6.88±0.17

Means inside the same column with different superscripts are different significantly at (p< 0.05) according to Duncan's multiple comparisons. R: Rejected samples.

Table 6: The mean counts ±S.E. (log₁₀ CFU/g) of *Enterobacteriaceae* in control untreated and treated chicken fillet samples (n = 3 for each group).

Treated groups Durations	Treated groups			
	Control	ozonized water	Lactic acid	Ozonized water - lactic acid blend
0.5 hour (Log reduction)		3.23 ± 0.12 ^b (0.73)	3.09 ±0.12 ^b (0.87)	3.18±0.13 ^b (0.78)
One hour (Log reduction)	3.96± 0.05 ^a	3.05 ± 0.03 ^b (0.91)	2.83±0.09 ^{bc} (1.13)	2.76 ±0.13 ^c (1.2)
2 hours (Log reduction)		2.95 ± 0. 03 ^b (1.01)	2.77±0.11 ^b (1.19)	2.57 ±0.13 ^c (1.22)

Means inside the same column with different superscripts are different significantly at (p< 0.05) according to Duncan's multiple comparisons.

Table 7: The mean counts ±S.E. (log₁₀ CFU/g) of the inoculated *S. aureus* in control untreated and treated chicken fillet samples (n = 3 for each group).

Treated groups Durations	Treated groups			
	Control	ozonized water	Lactic acid1%	Ozonized water - lactic acid blend
0.5 hour (Log reduction)		5.29 ± 0.20 ^b (0.82)	4.95 ±0.05 ^c (1.16)	4.82±0.10 ^c (1.29)
One hour (Log reduction)	6.11± 0.10 ^a	4.49 ± 0. 12 ^b (1.62)	4.30±0.17 ^b (1.81)	4.13 ±0.04 ^b (1.98)
2 hours (Log reduction)		3.96 ± 0.03 ^b (2.15)	3.92±0.03 ^b (2.19)	3.47 ±0.23 ^c (2.64)

Means inside the same column with different superscripts are different significantly at (p< 0.05) according to Duncan's multiple comparisons.

DISCUSSION

Shelf life, which includes appearance, texture, flavor, color, and nutritional value, is the amount of time that passes between a product's packaging and consumption during which the product properties stay acceptable to the user of

product (Singh and Singh 2005). The meat and meat products shelf life's depends on the level of its microbial contamination. Thus, food microbiologists and technologists had critical objectives, including raising the keeping quality of meat and reducing or getting rid of food-borne infections and

spoiling agents (Okolocha and Ellerbroek 2005).

Sensory evaluation of chicken fillet through the period of refrigerated storage was cleared in table 1 and showed that sensory characteristics were affected by different methods of treatments like lactic acid and a blend of ozonized water and lactic acid, these were proven to be highly effective in delaying sensory changes in refrigerated chicken fillet and its shelf life extending and decomposed to 9th and 11th day respectively, meanwhile ozonized water alone extend the shelf life one day more than control samples. The sensory changes were attributed to proteolysis and lipid oxidation in untreated samples (control) that were more obvious in shorter time than those in treated samples due to progressive growth of microbial load, this specific spoilage fault is a result of an accumulation of extremely high numbers of microbial cells instead of being produced by any specific metabolic activity of the microorganisms. These results similar with Moghassem Hamidi *et al.* (2021) who found that chicken meat stored in a refrigerator had improved sensory properties due to the using of neutral electrolyzed water.

Regarding to the results cleared in the table 2 the initial pH values were almost identical in each group. The values of pH diminished immediately after treatment with lactic acid and lactic acid - ozonized water mixture, in comparison to control samples, similar to our findings Aktas *et al.*, 2003) declared that the lactic acid addition to the meat systems resulted in diminish the pH initial values. The pH mean values On the 3rd day increased gradually within cold storage at $4 \pm 1^{\circ}\text{C}$; however it remain acceptable, at the 5th day the untreated control samples had pH value (6.61 ± 0.024) higher than treated groups

(6.18 ± 0.015 , 6.02 ± 0.01 , 6.07 ± 0.015 , 6.14 ± 0.014) ozonized water, Lactic acid and ozonized water - lactic acid blend respectively, at the seventh and ninth day the pH value exceed the permissible limit, this is due to the breakdown of protein /lipid as a result of chemicals, physical and microbiological damage with formation of alkyl group and accumulation of ammonia. The initial spoilage occurs at pH 6.2 (Pearson, 2006). High pH value recorded at the 5th day of storage at control group was similar to that reported by Hernandez-Pimentel *et al.* (2020), declared that an increase in pH values in the chicken fillet samples (treated or control groups) at the sixth day throughout the refrigerator storage period. Slight reduction of the pH caused by organic acids is fulfilling to avoid the growth of several bacteria (Stratford and Anslow, 1998). Therefore, treatment with lactic acid and its mixture with Ozonized water stabilized the pH of the injected chicken breast.

The measurement of total volatile Basic nitrogen (TVB-N) is a traditional chemical mean it common used to assessment the degree of meat spoilage and it shouldn't over than 20 mg TVN/100 g according to (ESO, 2005/1651). The content of TVN in chicken point for evaluating the freshness of chickens as an important reference index, (Ozogul and Özogul, 2000). Results tabulated in table 3 measured normal values of (TVB-N mg %) in the untreated and treated samples until the day 5, we note that the average value of TVB-N in the untreated control samples (21.30 ± 0.36) is slightly higher than the permissible limits. and the treated samples continued to be within the normal range, on day 7 the TVB-N values was 22.26 ± 0.49 for ozonized water treated samples and the others still within normal values going to the ninth day it reach to 20.41 ± 0.33 and 21.63 ± 0.31 for

lactic acid and ozonized water -lactic acid blend treated groups respectively. This is almost in line with findings from Rukchon *et al.* (2011) that TVB-N was significantly found in fresh chicken and that its level increased with storage period, as well as findings from Khalafalla *et al.* (2016) that showed TVB-N at the third and sixth days of storage was significantly higher in the Control group than all treated groups. It's possible that microbial activity at low temperatures is the cause of this TVB-N rise (Ibrahim and Desouky, 2009). However, Shenouda (1980) described that the rise in TVB-N is not associated with microbiological activity, but rather is typically brought on by autolytic enzymes and deamination.

Trimethylamine (TMA), Ammonia and Dimethylamine (DMA) make up the majority of TVB-N compounds found in chicken, and their concentrations rise as the chicken spoils due to enzymatic or bacterial degradation (Khalafalla *et al.* 2016). According to Ndaw *et al.* (2008), TBA is a useful diagnostic for determining the quality of meat and the extent of oxidative rancidity and lipid oxidation. Malondialdehyde (MDA) is the main byproduct of oxidative rancidity and is responsible for the bad flavor of oxidized fat. It has been suggested that 0.9 mg/kg is the highest TBA value that denotes high-quality chicken meat (ESO, 2005/1651). As cleared in table 4, TBA mean values for all treated and untreated groups nearly the same and significant increase with the advanced storage till the 5th day. At the 7th day control untreated sample show undesirable increase which indicate spoilage, followed by ozonized water treated samples, meanwhile samples treated with lactic acid and the ozonized water -lactic acid blend still within the acceptable limit until the day ninth. According to obtained result, lactic acid alone or in combination with

ozonized water effectively prevented lipid oxidation in samples of chicken meat. These data support the findings of Lee *et al.* (2023) who found that lipid oxidation in chicken meats was inhibited by plasma activated organic acid. These results are in conflict with those of Alahakoon *et al.* (2014) who found that calcium chloride mixture with lactic acid or alone were ineffective in inhibiting lipid oxidation in meat samples. This disagreement with Muhlisin *et al.* (2015) how demonstrated that there was no significant difference in the TBARS values during first 2 days, for treated samples with ozone compared to control samples. While, at 3 days of storage ozone significantly increased lipid oxidation, this may be the consequence of ozone exposure impairing the function of antioxidant enzymes or direct ozone attack on cell lipids, leading to irreversible damage to cell membrane fatty acids. A relation between chemical parameters (pH, TVB-N and TBA) and sensory evaluation were seen in all control and treated groups telling that using of ozonized water- lactic acid blend improve the quality of chicken fillet to an extent. However, the study of their effect on bacteriological quality is still required.

Table 5 indicates a comparison of effectiveness of ozonized water, Lactic acid and Ozonized water - lactic acid blend used separately on microbial stability of the chicken fillet samples which stored at 4 °C. Regarding to treated samples, APC significantly reduced from the first day of treatment, Ozonized water - lactic acid blend was the variable that most significantly reduce the microbial growth followed by Lactic acid>ozonized water,. By the third and fifth day all treatment trials also showed a significant reduction in APC. Meanwhile at the 7th day signs of

deterioration appear on control samples and no further bacteriological evaluation could be done. At the same time the three treatment groups declared a delayed growth in APC till the day 7th by ozonized water and to the day 9 by Lactic acid and ozonized water - lactic acid blend. But the highest effect recorded by Ozonized water - lactic acid blend. According to Casas *et al.* (2021) and Rangel *et al.* (2021), one of the main causes of ozone's disinfecting power is its oxidation-reduction potential (2.08 eV) and rise in intracellular reactive oxygen species (ROS), which cause bacterial cell lysis and have a negative impact on nucleic acid. Similarly, According to Jindal *et al.* (1995), immersion of chicken drumsticks in ozonized water at 0.44 to 0.54 ppm for 45 minutes resulted in 1.11-log CFU/cm² reductions in aerobic plate counts. This in agreement with Karamah and Wajdi (2018) findings, which showed that when chicken fillets are contacted with ozonized water containing 0.38 mg/l of ozone for 120 minutes at 3°C, the amount of aerobic mesophilic bacteria is reduced by 1 unit log cfu/g. This is in line with the findings of Alahakoon *et al.* (2014) found that there were a significant reductions in the total aerobic counts of treated samples by calcium chloride that were combined with 0.002% or 0.01% lactic acid and untreated control samples.

Concerning to the impact of lactic acid, ozonized water and their mixture on *Enterobacteriaceae* group it was clear in Table (6) that they have a significant effect with a log reduction of 0.73, 0.87 and 0.78 after half hour, 0.91, 1.13 and 1.2 after one hour and 1.01, 1.19 and 1.22 after two hours of immersion in ozonized water, lactic acid and their combination respectively. Similarly Smaoui *et al.* (2012) reported that the reduction the counts of

Enterobacteriaceae reported for 0.9% sodium lactate /0.09% lactic acid mixture in marinated chicken, even though Saleh *et al.* (2022) proved that the reduction percentage values of total *Enterobacteriaceae* count with lactic acid 1%, were 41.6, 57.6 by 5 and 10 minutes respectively, Sharma and Hudson (2008) who discussed that at 25 ppm ozone reduced the number of 15 bacterial species both Gram negative and Gram positive bacteria by greater than 3 log cfu/ml. furthermore, Yang and Chen (2007) found that throughout the whole refrigerated period, the broiler parts treated with ozone had microbial counts that were lower than the control parts. According to the findings of EL Dahshan *et al.* (2013), after 9 days of storage, the treated groups that received 40, 60, and 70 ppm of ozone for 20 minutes remained within the permissible limits authorized (EOS-1090/2005); these indicated that the ozone treatment extended the shelf-life of the chicken breasts.

Table 7 illustrated that the three groups of treatments were significantly reduce the count of *S. aureus* artificially inoculated in the examined chicken fillet samples and arranged as follow Ozonized water - lactic acid blend > lactic acid > ozonized water with a log reduction of 2.64, 2.19 and 2.15 respectively after two hours and it was approved that ozonized water-Lactic acid blend for 2 hours is the trial of choice, this come in agreement with (Kazemi Taskooh *et al.*, 2016). But higher reduction percent obtained by Kanaan (2018) who recorded that MRSA levels had decreased by 2-4 log₁₀ CFU/ml following treatment with 0.5 ppm ozonated water, for 45 min, Bialoszewski *et al.* (2011) who demonstrated that when *S. aureus* was exposed to ozonated water for 30 seconds at low ozone concentrations (1.2–3.6 µg/mL), nearly total

eradication was observed. Furthermore, Song *et al.* (2018) they showed that 1 mg/L ozonated water had an effective sterilization to 100% *S. aureus* in one min. In contrary higher reduction percent of *S. aureus* by of lactic acid 1 % and 1.25% were 99.88% and 21.34 %, respectively this demonstrated by Edris *et al.* (2020) and Saad *et al.* (2015), respectively. Hecer *et al.* (2007) who compared the effects of 1.5 ppm O₃ and chlorine (30 ppm) for 7 minutes, the average effects on the number of Staphylococcus was 81.33%. Khadre *et al.* (2001) proved that 0.3 to 1.97 mg/mL aqueous ozone inactivated *S. aureus* by 4 to 6 log₁₀ CFU/mL.

CONCLUSION

It was declared that all three trials of the treatment especially combined ozonized water-Lactic acid treatment were effective in keeping the sensory and chemical quality of chicken fillet reducing the total count of microorganisms, *Enterobacteriaceae* count and *S. aureus* count in chicken fillet. Thus, their use individually or in combination is recommended in chiller water of slaughter house.

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تسليط الضوء على تأثير حمض اللاكتيك والماء المعالج بالأوزون على فترة صلاحية فيليه الدجاج

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تم تجميع بعض عينات فيليه الدجاج من مدينة الزقازيق بالشرقية. ثم قسمت العينات إلى ثلاث مجموعات المجموعة الأولى: لتقييم فعالية المواد المضافة على الصفات الحسية ومؤشر الجودة الكيميائية (pH, TBA, TVN) و APC للعينات المفحوصة فيما يتعلق بالعمر الافتراضي للعينات المعالجة. المجموعة الثانية: لتقييم فعالية المواد المضافة على عدد البكتيريا المعوية. المجموعة الثالثة: لتقييم فعالية المواد المضافة على المكورالعنقودي الذهبي المحقونة بتركيز 10^6 خليه / جم في عينات فيليه الدجاج وبالتالي قسمت كل مجموعة إلى 4 مجموعات فرعية (المجموعة الضابطة، المجموعة المعالجة بالماء المعالج بالأوزون 0.38%، حمض اللاكتيك 1% وخليط من الاثنين معا). وقد اتضح أنغم العينات في الماء المعالج بالأوزون وحمض اللاكتيك يمكن أن يطيل العمر الافتراضي لفيليه الدجاج أثناء التخزين المبرد عند درجة حرارة 4 درجة مع الحفاظ على الخصائص الحسية والمعايير الكيميائية ليوما اضافيا , كما أنه يمكن لمزيج كل من الماء المعالج بالاوزون وحمض اللاكتيك أن يطيل مدة الصلاحية إلى أربعة أيام اضافية. فيما يتعلق بتأثير المعالجات المختلفة على الميكروبات محل الدراسة فقد ثبت أن مزيج الماء المعالج بالأوزون وحمض اللاكتيك يقلل بشكل كبير من العدد الكلي للبكتيريا وعدد البكتيريا المعوية وعدد البكتيريا المكورالعنقودي الذهبي يليه الماء المعالج بالأوزون وحمض اللاكتيك , وبالتالي يوصى باستخدامه في المياه المبردة في مجازر الدواجن.