



Egyptian Journal of Animal Health

P-ISSN: 2735-4938 On Line-ISSN: 2735-4946
Journal homepage: <https://ejah.journals.ekb.eg/>

Effectiveness of citrox in controlling of *Staphylococcus aureus* in refrigerated chicken meat

Taghreed H. Abbas* and Shereen A. Yassin**

*Reference Lab for Safety Analysis of Food of Animal Origin, Food Hygiene Department, Animal Health Research Institute, Dokki, Agricultural Research Center (ARC), Giza, Egypt.

**Food Hygiene Department, Animal Health Research Institute, Kafr-Elsheikh branch, Dokki, Agricultural Research Center (ARC), Giza, Egypt.

Received in 10/1/2024
Received in revised from
14/2/2024
Accepted in 27/3/2024

Keywords:

Staph. Aureus
chicken fillets
Citrox
Shelf life

ABSTRACT

Chicken meat are easily contaminated with different microorganisms including spoilage and pathogenic bacteria, posing risks that may lead to health hazard for consumer. Employing innovative methods during food storage represents a novel approach to manage and mitigate these potential dangers. Hence, the primary objective of this research was to improve the shelf life and safety of refrigerated chicken fillets by employing citrox solution, it is a compound a mixture of citric, malic and ascorbic acids. Citrox solution used at three concentrations (1%, 2%, and 4%) to assess its antimicrobial effect against colonization of *S. aureus*, physico-chemical parameters (pH, total volatile nitrogen, and thiobarbituric acid), and the sensory attributes of chicken fillets stored at 4°C for 18 days. The findings indicated a significant reduction in *S. aureus* count by about 2 log_{cfu}/g with the addition of different concentrations of citrox solution. Also, citrox solution improved the physio-chemical properties of chilled chicken fillets (pH, TVB-N, and TBA) through its protection from deterioration. Moreover, improved the sensory attributes of examined samples of chilled chicken fillets compared with control one. Concentrated 4% of citrox solution had the optimal significant effect than 1% and 2%. In conclusion, the study suggested that utilizing citrox solution as a natural antibacterial and antioxidant preservative for chicken fillets stored at 4°C could extend its shelf life up to 18 days compared with control group which was completely spoiled at the 9th day of storage.

INTRODUCTION:

Food safety is an important goal for public health as well as the economy. Every year, about 1 in 10 people get food poisoning as a

result of consuming food contaminated with pathogenic microorganisms (Lee and Yoon 2021). Given the interconnection between food safety and nutrition for health outcomes from

Corresponding author: Taghreed H. Abbas, Food Hygiene Department, Animal Health Research Institute, Dokki, Agricultural Research Center (ARC), Giza, Egypt.

E-mail:

DOI: 10.21608/ejah.2024.351121

food systems (WHO 2022), the task of delivering high-quality, safe, and nutritious food is anticipated to become increasingly challenging in the next decades (EC 2020).

Consumers prefer chicken meat more than other edible meats in particular because of its distinctive qualities. In addition, having good nutritional value, chicken meat is regarded as a better protein source because it has less fat and cholesterol than other meat. During the preparation and processing of chicken, several food borne pathogens are more prone to contaminate the chicken meat. This microbial contamination may cause harm for public health that has a serious impact on healthcare costs in addition to maximizing economic and financial losses for the producer linked businesses and employees (Cavitte 2003). Most pathogens like *S. aureus*, *Salmonella* sp., *Listeria* sp., *Campylobacter* sp., and *Vibrio cholera* could be present in poultry meat (Gonçalves-Tenório et al. 2018). As a result of its biological characteristics and chemical composition, chicken meat is a highly perishable foods that serves as an excellent source for the growth of dangerous microorganisms that can infect humans, and cause meat spoilage and economic loss Lika (2021). Nonetheless, chicken meat products frequently contain *S. aureus*, a bacterium that causes food poisoning (Qian et al. 2022).

Staph. aureus is the major Gram-positive bacteria that have drawn attention because its associated hospital and community acquired infections (Bush and Bradford 2020). *Staph. aureus* is a serious threat in chicken meat since it is resistant to many antibiotics, including methicillin (Dehkordiet al. 2017). At room temperature, this bacterium grows rapidly and produces various types of enterotoxins that lead to food poisoning (Hennekinnem , 2018). Naturally, *S. aureus* is widely distributed across the world, although food is the main source of infection for this organism (Ebert, 2018). The most economically significant food borne illness is caused by staphylococcal food intoxication (Chen et al. 2020) which causes gastrointestinal illness by action of varieties of enterotoxins (Abril et al. 2020). *Staph. aureus*, resulting in vomiting and diarrhea within 2 to 6

hours after eating contaminated food with already performed enterotoxins (Ie et al. 2021). The kind of foods ingested on a daily basis that *S. aureus* may thrive in optimally varies from nation to country mainly due to regional differences in food consumption customs (Argudín et al. 2010; Kadariya et al. 2014). *Staphylococcus aureus* and other pathogens in meat are caused by poor hygienic practices applied during meat processing, as well as other flawed abattoir procedures like improper evisceration of animals, which increases the risk of gut pathogens contaminating meat (Jaja et al. 2020).

The primary concern associated with *S. aureus*, its capacity to generate enterotoxins (SEs). Among these toxins, SEA, SEB, SEC, and SED are particularly noteworthy as they account for 95% of food intoxication cases (Abdelghany et al. 2020). Additionally, SEs are heat stable toxins, which means they tolerate high temperatures and cannot be destroyed by regular cooking. Aycicek et al. (2005) noted that these toxins lack a unique flavor and appearance in food, making it difficult to detect them in food. As soon as 30 minutes have passed after consuming food contaminated with SEs, food poisoning ensues (Argudin et al. 2010).

The use of spices, essential oils (Eos), natural antimicrobials, and bio-preservative agents to improve food safety has drawn a lot of attention in recent years due to consumers' growing demand for fresh foods with longer shelf life (Petrou et al. 2012).

Due to the accelerated problems of staphylococcal food poisoning, resistance of *S. aureus* to some antibiotics, toxicity, high sensitivity and infection in last years, recent research has focused on using the natural antimicrobials as alternative to chemical preservatives in food to satisfy consumer demand for more natural food preservatives (Pisoschi et al. 2018) as concerns have arisen due to the use of the chemical preservatives.

Citrous essential oils (CEOs), are extracted from peel of citrous fruits, and considered the

most widely used Eos in the world (Tsiraki and Savvaidis 2016). Citrox comprises soluble bioflavonoids derived from citrus fruits, of plant origin containing citric, ascorbic, and malic acids. Citrox is a yellow color solution with pH 2.7. Citrox is a novel natural antimicrobial as the organic acids and the bioflavonoid compounds have synergistic antiviral, antibacterial (Gram-negative and Gram-positive bacteria) effects, it emerges as an alternative preservative due to its efficacy in the presence of organic matter, it disrupts biofilms, prolongs shelf life, mitigates pathogenic threats, thus can be directly incorporated into food as an food preservative (Vardaka et al. 2016). Because of these previous advantages, citrox solution can be used as a preservative and to help reduce the possibility of contamination of food of animal origin from antibiotic-resistant bacteria, and thus prevent its hazard to human health. Combination of two natural antimicrobials (citrox and oregano essential oil) has a great antimicrobial effect against spoilage M.Os (TVC, Enterobacteriaceae, mold and yeast, and pseudomonas) as well as, extends shelf life for 21 day under vacuum packaging at 4°C and for 11 day at 12°C in buffalo meat (Osaili et al. 2023).

So, the object of this study was to evaluate the effects of citrox on the viability and growth of *S. aureus* in refrigerated chicken meat and the sensory attributes of the chicken meat.

MATERIAL AND METHODS

Bacterial strains

The reference strain of *S. aureus* was obtained from Reference Lab for Safety Analysis of Food of Animal Origin, Food Hygiene Department; Animal Health Research Institute and used by (10^7 CFU/mL). To activate the strain, 1 mL overnight culture was introduced to 9 mL of Brain heart infusion broth (BHI, Oxoid CM 225, Basingstoke, UK) at 37°C for 24 h. Subsequently, the culture was then centrifuged at $13,400 \times g$ for 5 minutes to collect the sediments, which were subsequently washed twice with saline solution (0.85% NaCl) before preparing the final solutions. The bacterial concentration in each culture was assessed by plating 0.1 ml portions of suitably diluted culture

onto duplicate Baird Parker agar plates, which were incubated at 37 °C for 48 h. Active cultures containing bacterial density ranging from 1×10^6 - 1×10^7 cfu/ml was utilized.

Citrox solution preparation:

Citrox solution was prepared according to (Yehia et al. 2019). Citrox solution is a combination of citric, malic, and ascorbic acids, in 100 ml of sterilized water (18 g, 18 g, and 5 g) of each aforementioned organic acids respectively, was added and dissolved to obtain 100% concentration. This solution was diluted to 1, 2 and 4% by adding 1 or 2 or 4 ml up to 100 ml sterilized water. The pH was adjusted to 2.7. The citrox solution was sterilized for 15-20 minutes at 121 °C.

Antibacterial activity of citrox solution against *S. aureus*:

To evaluate the antibacterial efficacy of citrox, the standard double dilution method, as outlined in CLSI (2019) was employed. The minimum inhibitory concentration (MIC) of citrox against *S. aureus* was determined using serial double dilutions in tryptone soya yeast extract broth (TSYEB) medium. Two-fold dilutions of citrox were prepared to achieve final concentrations of (0.25, 0.5, 1.0, 2.0, 4.0 & 8.0) mg/ml with *S. aureus* concentration of 10^7 cfu/ml (adjusted by 0.5 McFarland's) in TSYEB (Himedia). Each tube was inoculated with a suspension of 100 µL from cfu/ml., while the control solely comprised inoculated broth, then the tubes were placed in the incubator at 37°C for 24 h. The MIC end point indicates the minimum concentration achieved when there is no apparent growth observed in the tubes. MIC of citrox solution was detected by lowest concentration of citrox solution that inhibits growth of *S. aureus* with lack of visible turbidity.

Sample preparation:

Total of 2000 g of fresh skinless, boneless chicken fillets were collected from local markets in Giza governorate. The samples were transferred directly to the Food Hygiene Department, Animal health research institute (AHRI) as soon as possible under completely sterile conditions. Inside laboratory, chicken

samples were divided into four equal groups (500 g each), chicken meat samples were aseptically cut into sections (5 cm × 5 cm) and dipped in phosphate buffer saline contained 2×10^7 cfu/ml of *S. aureus* strain for 2 minutes at room temperature. After the inoculation, the chicken fillet samples were kept at room temperature for 20 min to allow bacterial cell attachment. After that samples were divided into four equal groups:

First group control samples inoculated with *S. aureus* strain without any treatment.

Second group samples inoculated with *S. aureus* and treated by dipping in 1% citrox solution for 2 minutes.

Third group samples inoculated with *S. aureus* and treated by dipping in 2% citrox solution (concentration 2%) for 2 minutes.

Fourth group samples inoculated with *S. aureus* and treated by dipping in 4% citrox solution for 2 minutes.

All samples left to dry before packing in transparent multiple sterile labeled low density polyethylene bags, heat sealed, and stored at 4°C. *Staph. aureus* count was carried out every 3 days for successive three weeks during refrigerated storage.

Bacteriological examination of *S. aureus* count: According to FDA (2001).

Ten gm of each fillet sample group was weighted and mixed with 90ml buffered peptone water and homogenized in a sterile stomacher bag, one ml of the sample homogenate was serially diluted. One ml of each serial dilution was streaked on 3 plates of Baird Parker agar media (0.4, 0.3, and 0.3 ml) and let to dry, then incubated at 35°C for 48hrs. The experiment was performed in triplicate.

Physico-chemical analysis:

Measurement of pH: according to ES (63-11/2006):

50 g of samples from each chicken group were mixed with 200 ml of distilled water for a duration 2 min. Subsequently, the resulting supernatant was filtered, and 50 ml portion of the filtrate was further diluted with 50 ml of

distilled water. After thorough mixing for 10 min, the pH was measured using a pH meter (JENEWAY 3310).

Determination of total volatile basic nitrogen (TVB-N):

TVB-N was measured according to ES: 63-9/ (2006).

Determination of thiobarbituric acid reactive substances (TBARS):

The test relies on measuring of malonaldehyde (MDA) as the final product of lipid peroxidation, following the procedure outlined in ES: 63-10/ (2006).

Sensory evaluation:

Chicken fillet samples dipped in citrox solution (without bacterial inoculation) and a control sample (without bacterial inoculation and citrox solution) were roasted using a grill at high power for 10 min. A nine-point hedonic score was used to estimate sensory attributes of cooked chicken (color, odor, flavor, taste, and overall acceptability) by the ask panelists' staff members of Food Hygiene Department in Animal Health Research Institute. A 9-point hedonic scale was employed for evaluation where (like extremely received a score of 9, like moderately received a score of 5, dislike extremely received a score of 1).

Statistical analysis:

The measurements were replicated three times, and mean values ± Standard deviation (SD) were reported for each instance. The analysis of variance (ANOVA) was done and mean using of Statistical Packaging for the Social Science (SPSS) Ver. 20. A p-value less than 0.05 ($p \leq 0.05$) was considered statistically significant

RESULTS:

Table 1. Staph. aureus mean count (log 10 cfu/g ± SD) in control and treated samples with citrox solution during refrigeration at 4⁰C (log cfu/g):

Treatments	Zero	3 rd	6 th	9 th	12 th	15 th	18 th
Control	7.34±0.04 ^a	7.49±0.02 ^a	7.71±0.01 ^a	8.17±0.3 ^a	8.28±0.00 ^a	8.34±0.03 ^a	8.71±0.03 ^a
Citrox1%	6.96±0.01 ^b	6.62±0.02 ^b	6.50±0.01 ^b	5.85±0.01 ^b	5.71±0.01 ^b	5.49±0.02 ^b	5.44±0.01 ^b
Citrox2%	6.61±0.02 ^c	6.36±0.04 ^c	6.28±0.01 ^c	5.61±0.01 ^c	5.49±0.02 ^c	5.33±0.02 ^c	5.30±0.02 ^c
Citrox4%	6.36±0.05 ^d	6.12±0.01 ^d	6.02±0.02 ^c	5.34±0.03 ^d	5.15±0.01 ^d	5.02±0.03 ^d	5.00±0.03 ^d

There is a significance difference between means having different letters at the same day of storage (P < 0.5)

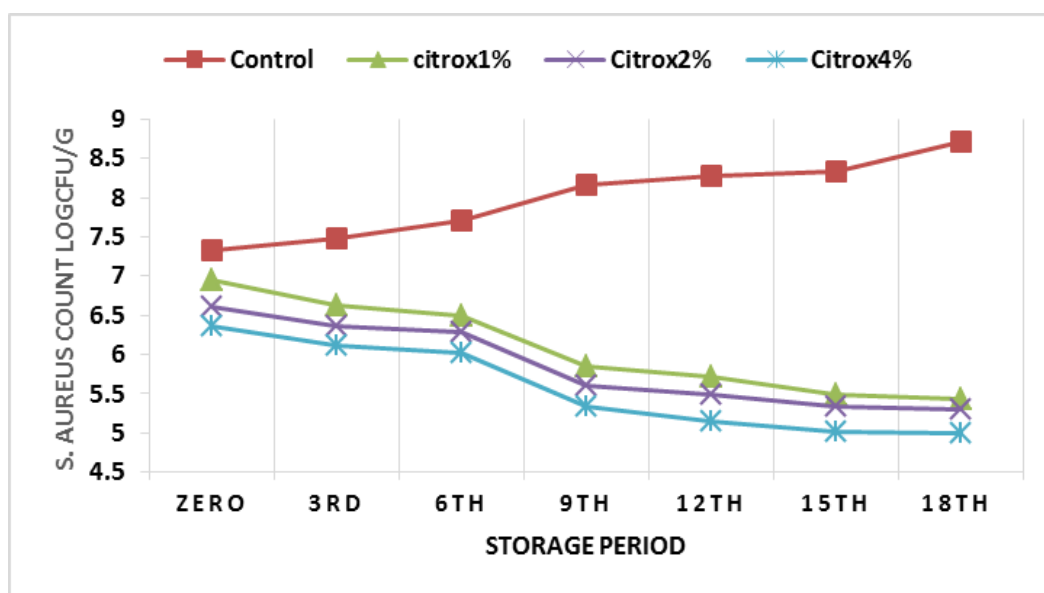


Figure 1. Means of *S. aureus* count (log_{cfu}/g. ± SD) in control and citrox solution treated groups

Table 2. Mean pH value of control and citrox solution treated groups:

Treatments	Zero	3 rd	6 th	9 th	12 th	15 th	18 th
Control	6.01±0.01 ^a	6.13±0.01 ^a	6.28±0.02 ^a	6.40±0.02 ^a	6.46±0.02 ^a	6.51±0.01 ^a	6.54±0.01 ^a
Citrox1%	6.01±0.02 ^a	6.12±0.01 ^a	6.21±0.02 ^b	6.33±0.01 ^b	6.38±0.01 ^b	6.42±0.01 ^b	6.46±0.01 ^b
Citrox2%	6.01±0.01 ^a	6.16±0.05 ^a	6.20±0.01 ^b	6.32±0.02 ^b	6.37±0.01 ^b	6.39±0.01 ^b	6.42±0.01 ^b
Citrox4%	5.87±0.01 ^b	6.07±0.01 ^b	6.12±0.01 ^c	6.14±0.01 ^c	6.18±0.02 ^c	6.22±0.01 ^c	6.33±0.01 ^c

There is significant difference between means having different letters at P < 0.05 for the same day of storage.

Table 3. Mean TVB-N value (mg/100ml) of the control and citrox solution treated groups:

Treatments	Zero	3 rd	6 th	9 th	12 th	15 th	18 th
Control	16.2±0.1 ^a	17.8±0.1 ^a	19.07±0.1 ^a	20.2±0.1 ^a	Spoiled	Spoiled	Spoiled
Citrox 1%	16.1±0.2 ^a	16.8±0.01 ^b	17.66±0.03 ^b	18.31±0.01 ^b	18.92±0.02 ^a	19.48±0.04 ^a	20.95±0.02 ^a
Citrox2%	16.06±0.1 ^a	16.32±0.02 ^c	17.45±0.02 ^c	18.41±0.01 ^c	18.54±0.002 ^b	19.43±0.02 ^a	20.89±0.02 ^a
Citrox4%	16.06±0.1 ^a	16.12±0.02 ^d	17.21±0.02 ^d	17.7±0.2 ^d	18.14±0.1 ^c	18.82±0.02 ^b	19.45±0.02 ^b

There is significant difference between means having different letters at p<0.05 for the same day of storage

Table 4. Mean TBA value (mg/kg) of control and citrox solution treated groups:

Treatments	Zero day	3 rd	6 th	9 th	12 th	15 th	18 th
Control	0.32±0.01 ^a	0.53±0.01 ^a	0.74±0.03 ^a	0.91±0.01 ^a	Spoiled	Spoiled	Spoiled
Citrox 1%	0.31±0.01 ^a	0.44±0.01 ^b	0.56±0.01 ^b	0.63±0.02 ^b	0.72±0.01 ^a	0.78±0.01 ^a	0.91±0.02 ^a
Citrox 2%	0.31±0.01 ^a	0.41±0.01 ^b	0.54±0.01 ^b	0.62±0.02 ^b	0.70±0.01 ^a	0.75±0.03 ^a	0.90±0.01 ^a
Citrox 4%	0.30±0.0 ^a	0.34±0.01 ^c	0.40±0.02 ^c	0.47±0.0 ^c	0.56±0.01 ^b	0.61±0.01 ^b	0.85±0.02 ^b

There is a significance difference between means having different letters at P <0.05 for the same day of storage

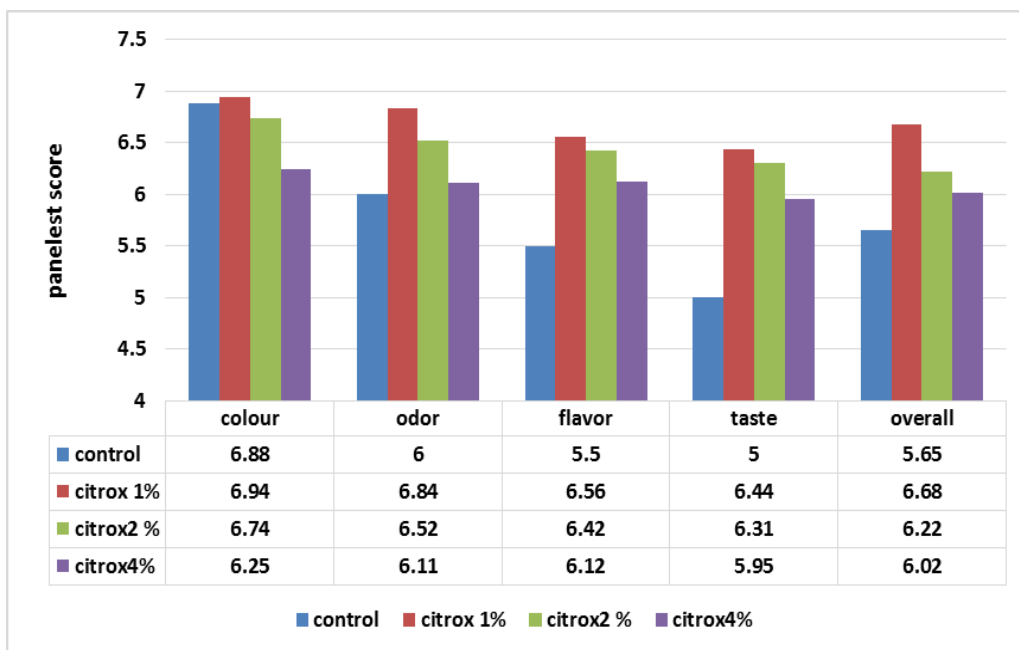


Figure 2. Sensory evaluation of citrox treated samples.

DISCUSSION:

Concerning the growing global consumption of chicken meat, consumers generally consider the safety of chicken meat should be a basic demand (Daghir et al. 2021). In this study, the antimicrobial activity of citrox solution against *staph. aureus* was evaluated by using the minimum inhibitory concentration "MIC" to measure the degree of its activity. Citrox solution proved to have antibacterial activity against *staph. aureus* at all concentrations mentioned before. So, through this challenge experiment, the antibacterial effect of citrox solution was assessed on chicken fillets that had been experimentally inoculated with *S. aureus*.

Table and fig. (1). Revealed the means counts of *S. aureus* ($\log_{10}\text{cfu/g}$) experimentally contaminating the raw chicken fillet samples during storage at 4 °C for 18 days and treated with different concentrations of citrox solution. The present data exhibited the potential of citrox solution as a natural food preservative against *S. aureus* in chicken fillet samples. At zero day, counts of *S. aureus* in chicken fillet samples after inoculation were 7.34 ± 0.04 , 6.96 ± 0.01 , 6.61 ± 0.02 and 6.36 ± 0.05 $\log_{10}\text{cfu/g}$ in control, 1%, 2% and 4% citrox solution treated group samples, respectively. By 3rd day of refrigerated storage period such counts of *S. aureus* slightly decreased to 6.62 ± 0.02 ; 6.36 ± 0.04 and 6.12 ± 0.01 $\log_{10}\text{cfu/g}$ after treatment with citrox solution 1%, 2% and 4% respectively, compared with the control group which recorded 7.49 ± 0.02 $\log_{10}\text{cfu/g}$. By the 6th day of storage, control group as well as 1, 2 & 3% citrox groups recorded 7.71 ± 0.01 , 6.50 ± 0.01 , 6.28 ± 0.01 and 6.02 ± 0.02 of *S. aureus* count. By the 9th day of refrigerated storage, the control sample group revealed a mean of 8.17 ± 0.3 $\log_{10}\text{cfu/g}$, while treated samples with citrox solution 1%, 2% and 4%, recorded 5.85 ± 0.01 ; 5.61 ± 0.01 and 5.34 ± 0.03 , By 12th day of refrigerated store, *S. aureus* count reduced to 5.71 ± 0.01 , 5.49 ± 0.02 and 5.15 ± 0.01 $\log_{10}\text{cfu/g}$, in treated groups with 1%, 2% and 4% citrox solution, while the count in control group was increased to record 8.28 ± 0.00 $\log_{10}\text{cfu/g}$. At the end of the 18th day of the experiment, the count of *S. aureus* in control and

treated samples with 1%, 2% and 4% citrox solution reached 8.71 ± 0.03 , 5.44 ± 0.01 , 5.30 ± 0.02 and 5.00 ± 0.03 , respectively.

There was a significant difference in the initial *S. aureus* count (day 0) between control and citrox treated groups ($p>0.05$). It was noted all over the experiment that the count of *S. aureus* in the control group at 4°C, significantly increased gradually ($p<0.05$). On the contrary, all citrox treated groups at 3rd, 6th, 9th, 12th, 15th, and 18th day of storage period exhibited significantly lower *S. aureus* count ($p<0.05$) while control group had elevated count. Also, the obtained results clarify that suppression of *S. aureus* count increased when the concentration of citrox are increased to 4% rather than 1% and 2% citrox treated groups. These results suggest that citrox solution was an effective factor in suppressing *S. aureus* population in chicken fillets, this antibacterial action may be due to citrox comprises a variety of bioflavonoids extracted from citrus fruits. Bioflavonoids are hydroxylated phenolic compounds synthesized by plants and have demonstrated efficacy against bacteria, fungi and viruses in prior studies. Both bioflavonoid and organic acid compounds display effectiveness against viruses, bacteria, molds, and yeasts and demonstrating synergistic interactions (Hooper et al. 2011; Yehia et al. 2021).

The obtained results in the present study were on line with Yehia et al. (2019) who reported that citrox is effective to reduce *S. aureus* (MRSA) count after three days of storage by achieving decrease in bacterial number by 1 \log_{10} cycle. Abdel-Naeem et al. (2022) noted that *S. aureus*, psychotroph, and Enterobacteriaceae counts were significantly ($p<0.05$) reduced by all fruit (citrus, grape and banana) peel powder-treated chicken patties when compared with untreated samples. Eldahrawy et al. (2022) indicated that the treatment of raw chilled minced beef with citrus peel powders exhibited a significant inhibition of *S. aureus* count compared to the control group at refrigerator temperature ($4\pm 1^\circ\text{C}$). Furthermore, Abu-Ghazaleh (2013) recorded that citric acid at concentration of 0.03% decreased the growth of *S. aureus* while ascorbic acid at concentration of 0.1% nearly completely inhibited the

growth of *S. aureus* in vitro.

Moreover, **Gonzalez-Fandos and Herrera (2013)** documented that applying of 2% malic acid treatment led to 1.14 to 1.83 log cycles reduction in total bacteria counts. Incorporating malic acid treatments in a hazard analysis critical control point (HACCP) strategy could enhance the microbiological safety and extend the shelf life of poultry meat, furthermore, the authors noted that malic acid treatment did not negatively impact the quality characteristics of poultry legs. **Eswaranandam et al. (2004)** stated that malic acid demonstrated greater efficacy in reducing pathogens compared to citric acid as a consequence of its molecular weight, which may facilitate its entry into the microbial cells. Ascorbic acid (vitamin C) is inexpensive, readily available, and has been documented for its antimicrobial effects against *S. aureus*, *Enterococcus faecalis* (**Isela et al. 2013**), *Mycobacterium tuberculosis* (**Vilch ze et al. 2013**), and *Aspergillus* spp. (**Verghese et al. 2017**). **Przekwas et al. (2020)** reported that employing vitamin C in the food industry might serve as an alternative approach to inhibit bacterial growth and eliminate biofilms. This is attributed to its capacity to reduce the pH in the environment, creating unfavorable conditions for bacterial survival.

The documented results in Table (2) showed that, the treated chicken meat groups with different concentrations of citrox had lower pH values than the control group. At zero day, a significant difference between the control and the citrox 4% treated group was significantly different (5.87 ± 0.01) as compared with the control as well as with treated citrox groups of 1% & 2% (6.01 ± 0.01 , 6.01 ± 0.02 and 6.01 ± 0.01) respectively, which may be attributed to the marinating chicken meat in citric acid could be dramatically lowered the pH that reduces microbial load (**Meltem et al. 2017**). From the 3rd to the 18th day of storage, citrox 1&2% treated groups not revealed any significant difference. While, citrox 4% treated samples were significantly lower than the control as well as 1&2% citrox treated groups, also the pH values increased in all treated groups in a slow rate (6.46 ± 0.01 , 6.42 ± 0.01 and 6.33 ± 0.01) in comparison with the control

group (6.54 ± 0.01) at the end of storage period (18 day) which inhibited the growth of most bacteria in chicken meat during chilled storage and demonstrating an antimicrobial effect of citrox solution. The aforementioned data explained that control group was unacceptable after 9th day of storage as the meat considered unfit for human consumption if the pH exceeded 6.4 as it mentioned by **Gracey and Collins (1992)**. In this respect, **Karabagias et al. (2011)** stated that amines and NH_3 (the alkaline reaction chemicals) are produced when protein breaks down into free amino acids, indicating the extent of meat spoilage causing an elevation of pH of the control chicken meat. The obtained results were similar to **Yehia et al. (2019)** who recorded that Chicken treated with 1% and 2% citrox solutions displayed an increase in pH level, but this increase was lower as compared to that of control group.

Enzymatic processes and microbial activity contribute to the degradation of Proteins in meat products, leading to the generation of TVB-N. Monitoring the TVB-N value serves as a crucial indicator for freshness and prolonging the shelf life of meat products (**Sun et al. 2021**). The TVB-N values of both the control and the treated groups with citrox solution are included in Table (3). TVB-N values for all groups exhibited a gradual increase during storage but it was substantially slower and suppressed through the preservation of all treated samples in comparison to the control sample. The rise in TVB-N level in chicken fillets may be due to autolytic degradation of nucleotides and free amino acids, microbial and autolytic processes, as well as complete microbial reduction of trimethylamine oxide to trimethylamine (**Duran and Kahve 2020**). At zero day, there was no significant difference between control and all treated groups recorded means \pm SD of (16.2 ± 0.1 , 16.1 ± 0.2 , 16.06 ± 0.1 , and 16.06 ± 0.1). A sharp increase of the control samples was noticed till spoilage by day 9th of storage with a mean of 20.2 mg/kg. Meanwhile, citrox solution treated samples (1, 2 and 4%) showed lower values (18.31, 18.41 and 17.7 mg/kg), respectively. There was a notable decrease in TVB-N levels in the citrox treated groups compared to the control group. Among the 1%, 2% and 4% citrox treated groups, cit-

rox 4% demonstrated optimal effect than other two concentrations. As by the 18th day, Citrox 4% revealed a mean of 19.45 ± 0.2 , while 1, 2% citrox treated samples recorded mean of (20.95 ± 0.02 and 20.89 ± 0.02), respectively. This lower significant rate in TVB-N may be attributed to presence of organic acids (citric and malic) which can potentially decrease the presence of spoilage and pathogenic bacteria by elevating of food products acidity (**Olaimat et al. 2018**). Citric acid demonstrates effective antimicrobial activity aiding in preservation of food combating bacterial spoilage (**Deepa et al. 2011**). The control group exceeded the permissible limit of TVB-N (20 mg/100 g meat) at the 9th day of storage and considered unacceptable according to (**ES 2005**), spoilage of 1,2% citrox solution treated samples were noticed at the 18th day of storage, in contrast, TVB-N levels in the 4% citrox solution treated group was within the permissible limit still the end of the 18th day, which showed a high significant difference between all treated and control groups. These results were in accordance with **Saleh et al. (2022)** who found a noticeable decrease in TVB-N level of chicken meat dipped in various concentrations of acetic acid, lactic acid, and lemon juice), and complied with those obtained by (**Yehia et al. 2019**) who reported that citrox was effective in decrease of TVB-N level of chicken meat.

Table (4) illustrated the variations in TBA values for the control and citrox treated groups during refrigeration storage. chemical deterioration through lipid oxidation is a primary factor constraining the shelf life of meat products and TBA serves as a valuable metric for evaluating the extent of lipid oxidation during food storage. Malondialdehyde (MDA) is a degradation product of lipid oxidation not only influenced the quality, but also has harmful effects on the human health, this product considered as a carcinogenic factor in food (**Djenane and Roncales, 2018**), At zero day, all groups started with low TBA values ranged from 0.30 ± 0.0 to 0.32 ± 0.01 MDA/kg and revealed no significant differences between the control group and treated groups. However, significance difference ($p < 0.05$) was clear between the control and 4% citrox treated groups by the 3rd day till the end of the storage period. By 6th day, no

significant difference was noticed between 1 and 2% concentrations of treated citrox groups (0.56 ± 0.01 & 0.54 ± 0.01). Meanwhile, the control group was significantly different from the citrox 4% group recording (0.74 ± 0.03 and 40 ± 0.02) MDA/kg, respectively, whereas all treated groups had low TBA level compared to control group and were within the permissible level reported by (**ES 2005**) where the limited value of TBA was not more than 0.9 mg of MDA/kg for chicken meat. On the 12th day of storage, the TBA values of all citrox treated groups increased to 0.72 ± 0.01 , 0.70 ± 0.01 and 0.56 ± 0.01 mg of MDA/kg while the control group had spoiled. Furthermore, lower significance values for TBA were observed between treated group with 4% citrox and both 1% and 2% citrox treated groups throughout the storage period. Also, it was clear that elevation of TBA level was rapidly in control group. This phenomenon could be attributed to rapid growth of microbes resulting in lipid oxidation during storage.

Over the storage duration, the TBA levels of citrox treated groups increased slowly until the 18th day, where the 1% treated samples had exceeded the permissible limit revealed means \pm SD of 0.91 ± 0.02 while, 2 % citrox group was stand at the margin of acceptance (0.90 ± 0.01), meanwhile, the 4% citrox treated group still sound till the 18th day of storage which recorded a mean \pm SD of 0.85 ± 0.02 . This may be attributed to the addition of citrox which contains ascorbic acid that used as natural antioxidant retard lipid oxidation of chicken meat. These results corroborate those recorded by other authors, who have used ascorbic acid to inhibit lipid oxidation in poultry meat during store (**Zahid et al. 2019; Ozer and Sariçoba 2010; Abou-Arab and Abu-Salem 2010**). All over the experimental period, it was noticed that 4% citrox solution showed the best values and maintained the shelf life of chicken meat till the 18th day, and was significantly higher than of the control, 1, and 2% citrox treated groups.

Furthermore, the outcomes of the current study proved that chicken fillets treated with different concentrations of citrox has acquired higher sensory scores for color, odor, flavor,

taste and overall acceptability which was showed in figure (2). means of 6.88, 6, 5.5, 5 and 5.65 for control group; 6.94, 6.84, 6.56, 6.44 and 6.68 for citrox 1%; 6.74, 6.52, 6.42, 6.31 and 6.22 for citrox 2%.

Furthermore, citrox 4% recorded means of 6.25, 6.11, 6.12, 5.95 and 6.02, respectively. Addition of citrox had a blend of flavorings and antioxidants property that could enhance both the nutritional value and sensory characteristics of the treated samples. All panelists' staff members approved that the solution reduced the hardness, enhanced the tenderness of the chicken fillets, and acquired the chicken fillets a spicy, fruity, and oriental flavors (citrus-like flavor) in comparison with the control group.

The highest sensory scores and overall desirability were more pronounced in chicken fillets with 1% citrox solution, followed by 2% and finally 4% citrox solution, these results were in line with **Yehia et al. (2019)** who stated that citrox enhanced the sensory items of chicken fillets, also prolonged their shelf- life to 21 days under vacuum packaged refrigeration storage. In this context, **Augustyńska-Prejsnar et al. (2023)** approved that the utilization of acidic fruit juices improved sensory attributes, particularly by diminishing hardness and enhancing the tenderness of the meat. **Unal et al. (2022)** stated that marinating chicken breast meat with citric acid (0.5%), lemon (100%) and grapefruit (100%) juices generally received higher scores from panelists compared with the control one. In this regard, **Serdaroğlu et al. (2007)** reported that marinating of turkey breast meat slices using different concentrations of citric acid and grapefruits increased juiciness and tenderness scores.

CONCLUSION:

From the overall obtained aforementioned data, it was concluded that citrox solution had improved bacterial, chemical and sensory quality of chicken fillet samples in comparison with the control group, and extend shelf- life of chicken meat. All the different citrox solution concentrations applied had a good potential as an antibacterial and satisfactory sensory characteristics of chicken fillet

especially at 4% which was the most effective and recommended. Additional studies should be carried out to discover the other benefits of citrox solution as a natural food preservative and disinfectant agent with antibacterial activities.

REFERENCES:

- Abdelghany HA, Zaki HM, Tolba KS, Yassin NA. 2020. Staphylococcus aureus and enterotoxin a relative gene expression in beef meat after lactic acid treatment and storage at different temperatures. *Bulg J Vet Med* 23(3): 378-388. Doi:10.15547/bjvm.2019-0007.
- Abdel-Naeem HH, Elshebrawy HA, Imre K, Morar A, Herman V, Paşcalau, R, Sallam KI 2022. Antioxidant and antibacterial effect of fruit peel powders in chicken patties. *Foods*, 11 (3)L301. doi:10.3390/foods11030301.
- Abou-Arab EA, Abu-Salem FM. 2010. Effect of natural antioxidants on the stability of ostrich meat during storage. *Grasas Y Aceites*, 61(1): 102-108. doi:10.3989/gya.042909.
- Abril GA, Villa GT, Barros-Velázquez J, Cañas B, Sánchez-Pérez A, Calo-Mata P, Carrera M. 2020. Staphylococcus aureus exotoxins and their detection in the dairy industry and mastitis. *Toxins*, 12(9):537. doi:10.3390/toxins12090537.
- Abu-Ghazaleh BM. 2013. Effects of ascorbic acid, citric acid, lactic acid, NaCl, potassium sorbate and Thymus vulgaris extract on Staphylococcus aureus and Escherichia coli. *Afr J Microbiol Res.*, 7(1): 7-12. Doi:10.5897/AJMR12.042.
- Argudín MÁ, Mendoza MC, Rodicio MR. 2010. Food poisoning and Staphylococcus aureus enterotoxins. *Toxins (Basel)*, 2 (7):1751-1773. doi: 10.3390/toxins2071751.
- Augustyńska-Prejsnar A, Kačániová M, Ormian M, Topczewska J, Sokołowicz Z. 2023. Quality and Microbiological Safety of Poultry Meat Marinated with the Use of Apple and Lemon Juice. *Int. J. of Enviro.*

- Res. and Public Health, 20(5): 3850. doi:10.3390/ijerph20053850.
- Aycicek H, Cakiroglu S, Stevenson TH. 2005. Incidence of *Staphylococcus aureus* in ready-to-eat meals from military cafeterias in Ankara, Turkey. *Food Cont.*, 16(6): 531-534. Doi: 10.1016/j.foodcont.2004.04.005.
- Bush K, Bradford PA. 2020. Epidemiology of β -lactamase-producing pathogens. *Clin. Microbiol. Rev.*, 33(2): 10-1128. doi:10.1128/CMR.00047-19.
- Cavitt JC. 2003. Present and future control of food-borne pathogens in poultry; revision of the European Community legislation on zoonoses. *Proceedings of the XVI European Symposium on the Quality of Poultry Meat. Saint-Brieuc (1): 46-58.*
- Chen J, Lü, Z, An Z, Ji P, Liu X. 2020. Antibacterial activities of sophorolipids and nisin and their combination against food-borne pathogen *Staphylococcus aureus*. *Euro. J. of Lipid Sci and Techno*, 122 (3): 1900333. doi:10.1002/ejlt.201900333.
- CLSI. 2019. Auto-verification of medical laboratory results for specific disciplines. Eighth Ed. CLSI guideline M07-A8. Wayne, PA: Clinical and Laboratory Standards Institute.
- Daghir N, Diab-El-Harake M, Kharroubi S. 2021. Poultry production and its effects on food security in the Middle Eastern and North African region. *J. of Applied Poult. Res*, 30(1): 100-110. doi: 10.1016/j.japr.2020.10.009.
- Deepa C, Jeyanthi GP, Chandrasekaran D. 2011. Effect of phytase and citric acid supplementation on the growth performance, phosphorus, calcium, and nitrogen retention on broiler chicks fed with low level of available phosphorus, *Asian J. of Poul. Sci*, 5(1): 28-34. Doi:10.3923/ajpsaj.2011.28.34.
- Djenane D, Roncales P. 2018. carbon monoxide in meat and fish packaging: Advantages and limits. *Foods* 7(2): 12 doi:10.3390/foods7020012.
- Dehkordi FS, Gandomi H, Basti AA, Misaghi A, Rahimi E. 2017. Phenotypic and genotypic characterization of antibiotic resistance of methicillin-resistant *Staphylococcus aureus* isolated from hospital food. *Antimicrob. Resist. and Infect. Control*, 6(1): 1-11.
- Duran A, Kahve HI. 2020. The effect of chitosan coating and vacuum packaging on the microbiological and chemical properties of beef. *Meat Sci.* (162):107961. Doi: 10.1016/j.meatsci.2019.107961.
- Ebert M. 2018. Hygiene principles to avoid contamination/cross-contamination in the kitchen and during food processing. In *Staphylococcus aureus* (pp. 217-234). Academic Press.
- EC. 2020. (European Commission) Farm to Fork Strategy for a Fair, Healthy and Environmentally-Friendly Food System. Communication from the EU Commission, COM, 381.
- Eldahrawy M, Salem AM, Nabil M. 2022. The efficiency of citrus peel powders in improvement of meat quality during chilled storage. *Benha Veterinary Medical Journal*, 42(2): 208-213. doi: 10.21608/BVMJ.2022.144967.1535.
- ES (Egyptian Organization for Standardization) 2006. Methods of analysis and testing for meat. Part 9: determination of total volatile nitrogen (TVN). ES:63-9/2006.
- ES (Egyptian Organization for Standardization) 2006. Methods of analysis and testing for meat. Part 11: determination of pH ES:63-11/2006.
- ES (Egyptian Organization for Standardization) 2006. Methods of analysis and testing for meat. Part 10: determination of thiobarbituric acid (TBA). ES: 63-10/2006.
- ES 1090/2005. Standards of poultry meat products, Ministry of industry, Cairo, Egypt. For frozen poultry and rabbit (2005).
- Eswaranandam SN, Hettiarachchy S, Johnson MG. 2004. Antimicrobial activity of citric, lactic, malic or tartaric acids and nisin

- incorporated soy protein film against *Listeria monocytogenes*, *Escherichia coli* O157:H7 and *Salmonella gaminara*. *J. Food Sci.* (69):79–84. Doi:10.1111/j.1365-2621.2004.tb13375.x.
- Food and drug Administration “FDA”. Detection and enumeration of *Staphylococcus aureus* in food. Bacteriological analytical manual. (2001; 8th ED. Chapter 12. Gaithersburg, pp.562.
- Gonzalez-Fandos E, Herrera B. 2013. Efficacy of malic acid against *Listeria monocytogenes* attached to poultry skin during refrigerated storage. *Poult Sci*, 92(7): 1936-1941. doi:10.3382/ps.2012-02968.
- Gonçalves-Tenório A, Silva BN, Rodrigues V, Cadavez V, Gonzales-Barron, U. 2018. Prevalence of pathogens in poultry meat: a meta-analysis of European published surveys. *Foods*, 7(5): 69.
- Gracey JF, Collins DS. 1992. Meat Hygiene. 9th Ed., Baillier, Tindall, England.
- Hennekinne JA. 2018. *Staphylococcus aureus* as a leading cause of foodborne outbreaks worldwide. In *Staphylococcus aureus* (pp. 129-146). Academic Press. doi:10.1016/B978-0-12-809671-0.00007-3.
- Hooper SJ, Lewis MAO, Wilson MJ, Williams DW. 2011. Antimicrobial activity of Citrox® bioflavonoid preparations against oral microorganisms. *British Dental Journal*, 210(1), E22-E22.
- Isela NR, Sergio NC, Martínez-Sanmiguel JJ, Hernandez-Delgadillo R, Cabral-Romero C. 2013. Ascorbic acid on oral microbial growth and biofilm formation. *The Pharma Innovation*, 2(4, Part A), 103. www.thepharmajournal.com
- Jaja I F, Jaja CJ, Chigor NV, Anyanwu MU, Maduabuchi EK, Oguttu JW, Green E. 2020. Antimicrobial resistance phenotype of *Staphylococcus aureus* and *Escherichia coli* isolates obtained from meat in the formal and informal sectors in South Africa. *Biomed Res Int.*, volume 2020I Article ID 3979842. Doi:10.1155/2020/3979482.
- Kadariya J, Smith TC, Thapaliya D. 2014. *Staphylococcus aureus* and staphylococcal food-borne disease: an ongoing challenge in public health. *Biomed Res. Int.*, doi: 10.1155/2014/827965. Epub 2014 Apr 1.
- Karabagias I, Badeka A, Kontominas MG. 2011. Shelf-life extension of lamb meat using thyme or oregano essential oils and modified atmosphere packaging. *Meat Sci.*, 88 (1):109-116. Doi: 10.1016/j.meatsci.2010.12.010.
- Le HHT, Dalsgaard A, Andersen PS, Nguyen HM, Ta YT, Nguyen TT. 2021. Large-scale *Staphylococcus aureus* foodborne disease poisoning outbreak among primary school children. *Microbiology Research*, 12(1): 43-52.
- Lee H, Yoon Y. 2021. Etiological agents implicated in foodborne illness worldwide. *Food Sci. Of Anim Resour.*, 41(1): 1-7. doi:10.5851/kosofa.2020.e75.
- Lika E. 2021. Sustainable Rural Development in Albania through Agriculture and Livestock: Challenges in the European Union Perspective. *J. Agron. Technol. Eng. Manag*, 4(2): 577-582. www.fimek.edu.rs/jatem.
- Meltem S, Kyialbek A, Alper O. 2017. The effects of marinating with citric acid solutions and grapefruit juice on cooking and eating quality of Turkey breast. *J. of Muscle Foods*. (8): 162-172. Doi:10.1111/j-1745-4573.2007.00074.
- Olaimat AN, Al-Holy MA, Abu Ghoush MH, Al Nabulsi AA, Qatatsheh AA, Shahbaz HM, Holley RA. 2018. The use of malic and acetic acids in washing solution to control *Salmonella* spp. on chicken breast. *J. of Food Sci.*, 83(8): 2197-2203. doi: 10.1111/1750-3841.14286.
- Ozer O, Sariçoba NC. 2010. The effects of butylated hydroxyanisole, ascorbic acid, and α -tocopherol on some quality characteristics of mechanically deboned chicken patty during freeze storage. *Czech J. of Food Sci.*, 28(2): 150-160. http://

- cabidigitallibrary.org by 156.200.180.201, on 02/24/24.
- Petrou S, Tsiraki M, Giatrakou V, Savvaidis IN. 2012. Chitosan dipping or oregano oil treatments, singly or combined on modified atmosphere packaged chicken breast meat. *International Journal of Food Microbiology*, 156(3): 264-271. <https://doi.org/10.1016/j.ijfoodmicro.2012.04.002>.
- Pisoschi AM, Pop A, Georgescu C, Turcuş V, Olah NK, Mathe, E. 2018. An overview of natural antimicrobials role in food. *Euro. J. of Medic. Chem.*, (143): 922-935. doi: 10.1016/j.ejmech.2017.11.095.
- Przekwas J, Wiktorczyk N, Budzyńska A, Walecka-Zacharska E, Gospodarek-Komkowska E. 2020. Ascorbic acid changes growth of food-borne pathogens in the early stage of biofilm formation. *Microorganisms*, 8 (4): 553. doi:10.3390/microorganisms8040553.
- Qian C, Castañeda-Gulla K, Sattlegger E, Mutukumira AN. 2022. Enterotoxigenicity and genetic relatedness of *Staphylococcus aureus* in a commercial poultry plant and poultry farm. *Inter.J. of Food Microbiol.*, 363, 109454. Doi: 10.1016/j.ijfoodmicro.2021.109454.
- Osaili T, Savvaidis IN, Atoui A, Tsiraki MI, Karam L. 2023. Microbiota and sensorial attributes of buffalo meat stored under vacuum packaging combined impact of marination, citrox and oregano essential oil. *Inter. J. of food proper.*, 26(1): 1953-1967. Doi: 10.1080/10942912.2023.2238919.
- Saleh, E, El-Morshdy AE, Aboelmkarm M. 2022. Improvement of the Chemical and Microbial Quality of Marketed Chicken Meat Using Organic Acids. *Damanhour J. of Vet. Sci.*, 8 (2): 16-21. doi:10.21608/djvs.2022.262814.
- Serdaroğlu M, Abdraimov K, Oenenc A. 2007. The effects of marinating with citric acid solutions and grapefruit juice on cooking and eating quality of turkey breast. *J. of Muscle foods*, 18(2): 162-172. doi: 10.1111/j.1745-4573.2007.00074.
- Sun K, Wang S, Ge Q, Zhou XI, Zhu J, Xiong G. 2021. Antimicrobial and preservative effects of the combinations of Nisin, tea polyphenols, rosemary extract, and chitosan on pasteurized chicken sausage. *J. of Food Prot.*, 84(2): 233-239. doi:10.4315/JFP-20-240.
- Tsiraki MI, Savvaidis IN. 2016. The effect of citrus extract (Citrox) on the naturally occurring microflora and inoculated pathogens, *Bacillus cereus* and *Salmonella enterica*, in a model food system and the traditional Greek yogurt-based salad Tzatziki. *foofMicrobiol.*, (53): 150-155. Doi: 10.1016/j.fm.2015.09.015.
- Unal K, Alagöz E, Çelik İ, Sarıçoban C. 2022. Marination with citric acid, lemon, and grapefruit affects the sensory, textural, and microstructure characteristics of poultry meat. *British poult.Sci.*, 63(1): 31-38. doi:10.1080/00071668.2021.1963674.
- Vardaka VD, Yehia HM, Savvaidis IN. 2016. Effect of citrox and chitosan on the survival of *E.coli* 0157:H7 and *Salmonella enterica* in vacuum-packaged turkey meat. *Food microbiol.*, (58):128-134. Doi:10.1016/j.fm.2016.04.003.
- Vilhèze C, Hartman T, Weinrick B, Jacobs Jr WR. 2013. *Mycobacterium tuberculosis* is extraordinarily sensitive to killing by a vitamin C-induced Fenton reaction. *Nature communications*, 4(1): 1881. doi:10.1038/ncomms2898.
- Verghese RJ, Mathew SK, David A. 2017. Antimicrobial activity of Vitamin C demonstrated on uropathogenic *Escherichia coli* and *Klebsiella pneumoniae*. *J. of Current Rese. in Scientific Medicine*, 3(2): 88-93. doi: 10.4103/jcrsm.jcrsm.35.17.
- WHO (World Health Organization) 2022. WHO global strategy for food safety 2022-2030: towards stronger food safety systems and global cooperation. World Health Organization.
- Yehia HM, Al-Masoud AH, Alsawmahi ON, Aljahani AH, El-Din MF. 2019. Effects of

citrox treatment on the survival of Methicillin-Resistant *Staphylococcus aureus* (MRSA) in chicken fillets packed under vacuum. *Food Sci. and Techno.*, (40): 588-595. doi: <https://doi.org/10.1590/fst.13819>.

Yehia HM, Al-Masoud AH, Elkhadragy MF, Korany SM, Nada HM, Albaridi NA, Al-Dagal MM. 2021. Improving the quality and safety of fresh camel meat contaminated with *Campylobacter jejuni* using citrox, chitosan, and vacuum packaging to extend shelf life. *Animals (Basel)*, 11(4), 1152. doi:10.3390/ani11041152.

Zahid MA, Seo JK, Parvin R, Ko J, Yang HS. 2019. Comparison of butylated hydroxytoluene, ascorbic acid, and clove extract as antioxidants in fresh beef patties at refrigerated storage. *Food Sci. of animal resources*, 39 (5): 768-779. Doi:10.5851/kosfa. 2019.e67.