Effectiveness of Ozone Treatment for Extending Chicken Burger Shelf Life

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Abstract

The aim of this research work was the determination of the efficiency of ozone treatment with different concentrations of ozone (600 ppm) during various exposure time periods (10, 20, 30, 40 min) in the prolongation of the shelf-life and maintenance of the quality of chicken burger during 15 days of storage under refrigeration conditions (4°C).

From the experimental results it can be concluded that ozone treatments resulted in a significant ($P<0.05$) decrease in the population of microorganisms and slowing down of proteolytic and lipolytic decomposition comparing to control samples, where spoilage (7.34 Log CFU/g) occurred rapidly along with alkalization (pH 6.90) by the 15th day. Although, the longer exposure time (30 and 40 min) provided the maximal reduction in the microorganisms' populations and successful stabilization of pH values, the significant increase in the content of the secondary lipid oxidation products (TBARS values reached 1.680 mg MDA/kg) occurred, which affected the sensory characteristics negatively (flavor, juiciness, acceptability).

On the contrary, moderate treatments (10, 20 min) helped to obtain the optimal balance. Thus, they prevented proliferation of microorganisms, stabilization of pH and keeping secondary lipid oxidation products (TBARS) in acceptable level (0.920 and 1.200 mg MDA/kg, respectively, by the 15th day). Besides, sensory evaluation showed that samples subjected to 10 and 20 min of ozonation had the highest sensory scores in such criteria as tenderness, appearance and overall acceptability, while minimizing off-odor formation. Thus, moderate ozone treatment (10-20 min) can be regarded as a highly promising residue-free hurdle technology.

Keywords: Ozone, Chicken burger, Shelf life extension, Thiobarbituric acid (TBARS), Microbial quality.

Introduction

Meat from chicken is considered to be a worldwide dietary component, which is famous for its high nutritional content and low price [46]. Specifically, chicken meat can be defined as the source of high-quality complete protein [25, 44], which contributes to muscle development and keeps bone mass [37]. Moreover, in comparison to red meat, chicken meat contains less fats and, thus, contributes to maintaining good health of the heart and aids in keeping healthy weight [11], while providing the body with vitamins and minerals.[36]

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However, chicken meat is highly perishable owing to its high water content and close-to-neutral pH, and, therefore, it is a perfect substrate for microbe growth .[42]

The problem becomes even more urgent in case of processed meats like burger where the mincing process increases the surface area for microbial contamination and speeds up the primary spoilage processes [33]. The latter include microbial spoilage caused mainly by Pseudomonas bacteria, which grow in a refrigeration temperature range and cause off-odors and slime development [31] as well as chemical spoilage, which refers to the oxidation of lipids resulting in rancidity [8] and proteins oxidation and deterioration of the nutritional value and meat texture [45]. In light of the increased concerns of consumers about synthetic preservatives [51], ozone treatment became a promising and safe alternative.[19 ,2 ,50]

Indeed, ozone is a strong oxidizer [32], which destroys bacteria, viruses, and fungi [13] through the destruction of their cell membranes. The main advantage of ozone consists in its quick transformation into oxygen without the leaving any toxic chemical traces [39]. Nevertheless, although the use of ozone as a food preservative has many positive aspects, it is a double-edge sword since its strong oxidative properties can contribute to the development of lipid oxidation in fresh meat.[35]

Taking into account the information provided above, the current paper is aimed to investigate the effect of the treatment of chicken burger with ozone treatment during 10, 20, 30, and 40 minutes on their microbiological, chemical, and sensory properties after packaging and 4°C refrigeration for 0, 5, 10, and 15 days.

Material and Methods

Preparation of Chicken Burger

Fresh, freshly slaughtered chicken meat samples were collected from local markets in Mosul, Nineveh, Iraq. Meat samples were brought to the laboratory on ice in an ice box with temperature 4 ± 1 °C in order to be microbiologically safe. The initial preparation started with manually skin removal and deboning, then trimming of fat, bruises and connective tissue from the meat to get maximum purity of the lean meat. The meat cuts were washed with chilled sterile water and dried with sterile paper towels. Finally, meat cuts were cut into cubes (2 x 2 cm).

Chicken meat cubes were minced using meat grinder with hole plate 4 mm in size, and this operation was repeated two times in order to reach maximum of uniformity of particle size. In the following step, a standard sample was prepared in amount of 1,000 g that consisted 80% of lean chicken breast and 20% lean chicken thigh. Then, 1,000 g of meat was weighed and added to the meat grinder with addition of 1.5% of salt. Kneading of the mixture was done during 3 min till obtaining homogenized slightly sticky batter.

This step included formation of 100 g meat samples that were formed into patties of equal size using analytical scale with subsequent molding by burger press of fixed dimension (10 cm in diameter and 1.5 cm in height) for equal surface area and volume of patty samples. Parchment paper was used for separation of individual patties from each other. Finally, samples were packed in plastic trays and plastic film bags. These samples were kept at temperature 4 °C in refrigerator until treatments application and initial measurements (day 0).

Ozone Treatment of Chicken Burger

The treated meat product (chicken burger) was subjected to ozone treatment at a level of 600 ppm for 10, 20, 30, and 40 minutes

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respectively. The ozonation treatment was carried out by direct immersion method using an ozone generator (Model HF-100, HeavenBird, China) , where the patties were immersed in distilled water that had been ozonized for the required period. The treated samples were kept at 4 °C temperature for 0, 5, 10, and 15 days.

Microbiological Analyses

Nutrient Agar, Skimmed Milk Agar, and Lipolytic Bacteria Media cultures were used and prepared according to the guidelines provided by their respective manufacturers. Each of the media was sterilized before use using an autoclave at 121 °C and 1 atm pressure.

Total Aerobic Bacterial Count

The aerobic bacterial count of the burgers was measured using the pour plate method on Nutrient Agar. The plates were then incubated at 37°C for 24 hours following the procedures detailed in [18, 12].

Total Proteolytic Bacterial Count

The number of proteolytic bacteria was enumerated via the spread plate technique as stated in [30]. This was performed on Skimmed Milk Agar containing 100 mL of Nutrient Agar and 10 mL of skim milk. Cultures were allowed to grow for 4-5 days at 28 °C. Proteolytic bacteria were identified by flooding the plate with 1% HCl for 1 min, after which it was washed using distilled water. A visible clear zone around the colonies indicated positive proteolytic bacteria.

Total Lipolytic Bacterial Count

The numbers of lipolytic bacteria were counted by means of spread plate technique [6] on Nutrient Agar with 1% oil and 1% Tween 80 medium. The plates were kept at 28 °C for 4–5 days. Afterwards, the colonies were checked for lipolytic activity by treating the medium with 20% CuSO4 solution for 5 minutes and then washed with distilled water. Blue-green colored colonies were considered lipolytic bacteria.

Determination of pH

pH measurement was carried out in accordance with [40]. Essentially, 5 g of the sample was ground in 45 mL of distilled water, and the resulting solution was filtered. The pH value of the filtrate was later measured by means of the Hanna HI98103 Checker pH meter.

Determination of Thiobarbituric Acid (TBARS) Value

The thiobarbituric acid (TBARS) value was measured by means of the colorimetric technique described by [16]. Chicken patty minced sample was mixed with 2.5 mL trichloroacetic acid solution in orthophosphoric acid solution and incubated for a specific period before adding distilled water. Filtration was performed using Whatman No. 1 filter paper. Three hundred microliters of the resulting solution was added to 0.3 mL of 0.005 M solution of thiobarbituric acid reagent, heated at 80 °C for 30 min, and then read on a spectrophotometer at wavelength of 532 nm. Absorbance obtained was multiplied by a constant factor of 7.8 in order to measure TBARS value in mg malondialdehyde/kg sample (mg MDA/kg).

Sensory Evaluation

Minced chicken patties were sensory evaluated after being fried for 6 minutes (3 minutes on each side). The panel assessed overall appearance, flavor, taste, juiciness, tenderness, off-odor intensity, and overall acceptability. Samples were randomly distributed on plates marked with three-digit random codes corresponding to each treatment. A 12-member panel was briefed beforehand on the scoring system using

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structured evaluation forms, and assessors rinsed their mouths with water between tastings, following the protocol of [17]. A 9-point Hedonic scale was applied to rate appearance, taste, flavor, juiciness, tenderness, and overall acceptability. Meanwhile, off-odor intensity was evaluated using an inverted 1–9 intensity scale (from best to least favorable), as outlined by [29]. Additionally, radar charts were utilized for data visualization following the method described by [27].

Statistical Analysis

Statistical analysis of the experimental data was conducted using a Factorial Completely Randomized Design (FCRD) with three replicates within the SAS [15]. Different letters indicate statistically significant differences between treatment means according to Duncan's Multiple Range Test at a significance level of $P < 0.05$.

Results and Discussion

As shown in **Table (1)** for the chicken burger samples refrigerated at 4°C over a 15-day period, all ozone exposure durations (10, 20, 30, and 40 min) induced varying significant reductions in the total aerobic bacterial count compared to the control at day 0. Specifically, 10-minute treatment yielded a value of 3.90 Log CFU/g, further decreasing to 3.78 and 3.70 Log CFU/g for 20 and 30-minute treatments, respectively, and reaching a minimum of 3.62 Log CFU/g at the 40-minute exposure, compared to the control which initially recorded 4.21 Log CFU/g.

Tracking the storage duration reveals that the aerobic bacterial counts in the control sample significantly increased ($P < 0.05$) over time, peaking at 7.34 Log CFU/g on day 15. In contrast, the ozone-treated samples consistently maintained lower values throughout the storage period compared to the control.

This variation highlights the efficacy of ozone in achieving an initial reduction in microbial load. Similarly, by the end of the storage period, this statistical advantage persisted, with values recording 6.07 (10 min), 5.24 (20 min), 5.11 (30 min), and 4.41 (40 min) Log CFU/g for the respective treatments values that remained significantly and markedly lower than the control (7.34 Log CFU/g).

However, when looking at the overall means through different durations of storage, one finds that the cumulative average definitely rises from 3.84 Log CFU/g on day 0 to 5.63 Log CFU/g on day 15. In contrast, looking at the overall means through different durations of exposure, one sees that there is definitely a decrease in microbial load; the average decreases from 5.65 Log CFU/g in the control to 4.81 (10 min), 4.43 (20 min), 4.33 (30 min), and even to 4.10 Log CFU/g in the 40-minute treatment group.

[49] , corroborate these results by affirming that aqueous ozone is an efficient sanitizer that ensures an immediate and continuous reduction of bacterial counts, thus confirming the decreasing values through all exposure durations.

[14] , Also support this, which considered ozone an ideal hurdle technology for poultry meat due to its ability to inhibit microbial growth while extending shelf life, explaining why the treatments maintained stable microbial values. In addition, these results correspond to the conclusions of [21], who proved that the persistent effect of ozone as a surface disinfectant causes consistent, statistically significant reduction in total bacterial numbers despite the long periods of storage.

Concerning other research papers dealing with the issue of microbiology, the study of [53] proved that the control of microbial

dynamics associated with sequential bacterial succession and the transition of dominance from initial lactic acid bacteria to aerobic spoilage Pseudomonas spp. is a key parameter of meat quality degradation. The findings of the current study prove their point of view that any early actions, like ozone treatment, can prevent from reaching peak microbial spoilage. This scientific interpretation correlates with the study of [3], who highlighted the significance of microbial inhibition techniques as a measure to reduce the risks of foodborne diseases, and stated that the ability of ozone to diffuse through cell membranes and inhibit bacterial enzymes is a key mechanism to avoid the development of pathogens.

As was mentioned by [14, 26], this high efficiency of the tested treatments can be explained by the oxidative mechanism of action of ozone. Oxidative molecules penetrate into the bacterial cell membrane destroying its selective permeability and causing the leakage of vital intracellular components. Besides, ozone treatment deactivates enzymes of the bacterial respiratory chain thus disrupting the intracellular energy transfer systems and inhibiting the growth of microorganisms during the storage period.

Table 1: Effect of ozone exposure durations (10, 20, 30, and 40 min) on the total aerobic bacterial count (Log CFU/g) of chicken burger samples stored at 4°C for 15 days.

Average	Storage Time at 4°C (days)				Ozone Exposure Time (min)
	15	10	5	0	
5.65 ± 0.337a	7.34 ± 0.009a	5.66 ± 0.032c	5.38 ± 0.035d	4.21 ± 0.041j	0
4.81 ± 0.242b	6.07 ± 0.012b	4.87 ± 0.028g	4.41 ± 0.020h	3.90 ± 0.026i	10
4.43 ± 0.158c	5.24 ± 0.018e	4.43 ± 0.015h	4.28 ± 0.015i	3.78 ± 0.024m	20
4.33 ± 0.153d	5.11 ± 0.020f	4.33 ± 0.007i	4.19 ± 0.013j	3.70 ± 0.012n	30
4.10 ± 0.091e	4.41 ± 0.003h	4.29 ± 0.007i	4.08 ± 0.009k	3.62 ± 0.026o	40
	5.63 ± 0.267a	4.72 ± 0.138b	4.47 ± 0.126c	3.84 ± 0.055d	Average

The results of the experiment performed on the basis of **Table (2)** of chicken burger samples stored in refrigerators at 4°C for 15 days prove the considerable effect of ozone treatment on the inhibition of the total activity of proteolytic bacteria. The statistical analysis of the average values depending on the duration of the exposure showed that with the increase of the exposure time there was a statistically

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significant (P<0.05) reduction in the rates of enzymatic activity; the average values were equal to 3.64 Log CFU/g in the control group and to 2.72, 2.62, 0.96, and 0.94 Log CFU/g in 10, 20, 30, and 40 minutes treatment, respectively. This inhibition effect of ozone correlates with the findings of [14, 21], who indicated that the physical ozone treatment has an antagonistic effect on the microbial enzymatic activity thus minimizing the protein degradation in foods.

Regarding the effect of refrigerated storage duration on proteolytic bacterial activity, the control sample exhibited initial microbial activity of 3.10 Log CFU/g on day 0, followed by a distinct and significant increase (P<0.05) over time, peaking on day 15 at 4.09 Log CFU/g. This direct effect of storage time is clearly reflected in the overall storage means in the same table, showing a continuous and significant rise in proteolytic bacterial activity across all samples as storage progressed, the overall mean increased from 0.62 Log CFU/g on day 0 to 1.96 and 2.19 Log CFU/g, ultimately reaching a maximum of 3.93 Log CFU/g at the end of the storage period. The microbial trends observed in this study are consistent with the findings of [48, 6], who reported that protease activity increases proportionally with extended storage in poultry meat products due to the growth and proliferation of enzyme-secreting bacteria.

Table 2: Effect of ozone exposure durations (10, 20, 30, and 40 min) on proteolytic bacteria count (Log CFU/g) in chicken burger samples stored at 4°C for 15 days.

Average	Storage Time at 4°C (days)				Ozone Exposure Time (min)
	15	10	5	0	
3.64 ± 0.115a	4.09 ± 0.032a	3.84 ± 0.035cd	3.52 ± 0.040fg	3.10 ± 0.100h	**0**
2.72 ± 0.483b	4.05 ± 0.058ba	3.63 ± 0.033ef	3.20 ± 0.100h	0.00 ± 0.000i	**10**
2.62 ± 0.466c	3.91 ± 0.063bc	3.48 ± 0.000g	3.10 ± 0.100h	0.00 ± 0.000i	**20**
0.96 ± 0.501d	3.84 ± 0.057cd	0.00 ± 0.000i	0.00 ± 0.000i	0.00 ± 0.000i	**30**
0.94 ± 0.490d	3.75 ± 0.050de	0.00 ± 0.000i	0.00 ± 0.000i	0.00 ± 0.000i	**40**
	3.93 ± 0.039a	2.19 ± 0.479b	1.96 ± 0.431c	0.62 ± 0.332d	**Average**

The interaction results between ozone exposure duration and storage time show a clear gradient in microbial inhibition efficacy, with treatments ranked progressively according to exposure time and their sustained effect through day 15 compared to the control (4.09 Log CFU/g). Accordingly, the 10-minute exposure had

a zero bacterial count at day 0 but showed 4.05 Log CFU/g after 15 days of storage. The 20-minute exposure started with zero bacterial count, but at the end of the storage period, there was an improvement of 3.91 Log CFU/g in bacterial count. In addition, the 30-minutes exposure exhibited zero bacterial count at the first three storage days but at day 15 recorded 3.84 Log CFU/g. The 40-minute exposure had zero bacterial count in the first 10 days of storage but the lowest value of 3.75 Log CFU/g in bacterial count at the end of the storage period.

The very high efficiency of Ozone in inhibiting proteolytic bacterial activity as noted in [14, 26] is linked to its oxidative mechanism of action. The oxidative molecules diffuse through the bacterial cell membrane and increase its permeability thus leading to leakage of vital intracellular compounds. Moreover, the oxidative molecules inhibit the protease enzymes involved in the respiratory chain and metabolic processes thus interfering with the intracellular energy transport system. Consequently, this leads to inhibition of microbial growth and proliferation.[28] Indeed, emphasized that reduction in the activity of proteolytic bacteria is necessary to preserve the sensory and structural characteristics of chicken burgers.

The results of statistical analysis shown in **Table (3)** for chicken burger samples stored under refrigeration at 4°C for 15 days suggest that ozone treatment plays an inhibitory role in suppressing lipolytic bacteria in chicken burgers. The results show that increasing the exposure time of the ozone led to a significant reduction ($P<0.05$) of these bacteria. It is seen from the fact that the overall mean in the control group was 3.845 Log CFU/g while the overall means after ozone exposure for 10, 20, 30, and 40 min were 3.023, 2.961, 2.020, and 1.940 Log CFU/g, respectively. This antimicrobial effect is in agreement with [48, 14] findings about the ability of ozone to inhibit microorganisms and enzymes in food products.

On the contrary, it is evident from the same table that increasing the storage period negatively affects these bacteria. The control group had the initial bacterial count of 3.20 Log CFU/g at the first storage day and then showed a statistically significant growth ($P < 0.05$). The maximum bacterial activity was observed at the last day of the storage period (day 15) with 4.48 Log CFU/g. This positive trend is confirmed in the continuous and proportional increase in the bacterial growth in the overall mean values during storage periods. In particular, it can be seen that the mean value increased from 0.64 Log CFU/g on the first day to 2.10 and 3.92 Log CFU/g, and reached a peak value of 4.37 Log CFU/g on the last storage day. The dynamics of these bacterial population are similar to the findings of [10, 34, 26] about the positive correlation between the lipolytic bacterial activity and the storage time in poultry meat.

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Table 3: Effect of ozone exposure durations (10, 20, 30, and 40 min) on lipolytic bacteria count (Log CFU/g) in chicken burger samples stored at 4°C for 15 days.

Average	Storage Time at 4°C (days)				Ozone Exposure Time (min)
	15	10	5	0	
3.845 ± 0.149a	4.48 ± 0.062a	4.10 ± 0.052c	3.60 ± 0.000e	3.20 ± 0.100g	**0**
3.023 ± 0.535b	4.44 ± 0.061a	4.09 ± 0.058c	3.56 ± 0.040e	0.00 ± 0.000h	**10**
2.961 ± 0.528b	4.42 ± 0.040a	4.06 ± 0.023c	3.36 ± 0.060f	0.00 ± 0.000h	**20**
2.020 ± 0.611c	4.27 ± 0.021b	3.81 ± 0.074d	0.00 ± 0.000h	0.00 ± 0.000h	**30**
1.940 ± 0.590d	4.24 ± 0.031b	3.52 ± 0.040e	0.00 ± 0.000h	0.00 ± 0.000h	**40**
	4.37 ± 0.031a	3.92 ± 0.063b	2.10 ± 0.460c	0.64 ± 0.343d	**Average**

The statistical interaction results revealed a clear gradient in the efficacy of ozone treatments throughout the storage period. The 40 minute treatment demonstrated the highest inhibitory efficacy, maintaining zero bacterial activity through day 5 and ending on day 15 with the lowest bacterial growth (4.24 Log CFU/g). It was closely followed in second place by the 30-minute treatment, which also maintained zero counts through day 5 before recording a very comparable bacterial activity of 4.27 Log CFU/g at the end of storage. Ranked third was the 20 minute treatment, which successfully inhibited bacterial growth completely (zero count) on day 0, after which its inhibitory effect gradually diminished, with bacterial activity rising to 4.42 Log CFU/g on day 15. This was immediately followed by the 10-minute treatment, which exhibited a similar pattern by starting with zero counts on day 0 and reaching 4.44 Log CFU/g on day 15. Finally, the control sample exhibited the highest continuous bacterial activity throughout the entire storage period, peaking at 4.48 Log CFU/g on day 15 a value significantly and substantially higher than all evaluated ozone treatments.

The effectiveness of ozone in inhibiting the proliferation and growth of lipolytic bacteria as described in [14, 26] results from its strong oxidative action. The oxidative compounds enter into the cell membrane of the bacteria and interfere with its selective permeability process, thus causing leakage of the essential substances inside the cells. In addition, this method of preservation causes inhibition of the lipase enzymes in the metabolic system of the bacteria, thus interfering with the transport of energy within the cell, thereby inhibiting the proliferation of microorganisms.

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The analysis results in **Table (4)** for chicken burger samples refrigerated at 4°C over a 15 day period showed significant differences ($P<0.05$) directly influenced by ozone exposure duration and storage time. The control sample exhibited a sharp upward increase, with pH values rising from 5.90 on day 0 to 6.90 on day 15, exceeding the normal range for chicken meat (5.96–6.07) reported by [24] and indicating product. In contrast, ozone treatments induced gradual stability in the chemical environment; the 10 minute treatment recorded an overall mean of 6.175, followed by the 20 minute treatment at 6.100, reaching the most effective treatments (30 and 40 minutes), which achieved the lowest overall means of 6.000 and 5.925, respectively.

This chemical stability is closely linked to the microbial analysis, as the elevation in pH in the control sample is attributed to the activity of psychrotrophic, proteolytic, and lipolytic bacteria. According to [9, 26] the accumulation of basic nitrogenous compounds (such as ammonia and amines) resulting from the enzymatic degradation by lipases and amylases is directly responsible for this increase. In comparison, the 40-minute ozone treatment successfully stabilized the pH at 6.10 by the end of the storage period, compared to 6.90 in the control sample, reflecting high inhibitory efficacy.

This stability is consistent with the findings of [54, 23] regarding the role of ozone as a pretreatment technology that disrupts microbial metabolic pathways and prevents proactive chemical changes associated with. Although some studies suggest that pH alone should not be relied upon as a sole indicator, the significant differences observed in the microbial assays confirm that ozone altered the microenvironment to suppress microbial proliferation. This agrees with the discussion by [20] and was further corroborated by [26] through their evaluation of volatile compounds and enzymatic activity as complementary evidence for assessing meat.

Table 4: Effect of ozone exposure durations (10, 20, 30, and 40 min) on pH values of chicken burger samples stored at 4°C for 15 days.

Average	Storage Time at 4°C (days)				Ozone Exposure Time (min)
	15	10	5	0	
6.400 ± 0.124a	6.90 ± 0.115a	6.60 ± 0.100b	6.20 ± 0.115cde	5.90 ± 0.115fg	**0**
6.175 ± 0.099b	6.60 ± 0.100b	6.30 ± 0.115cd	6.00 ± 0.058efg	5.80 ± 0.058g	**10**
6.100 ± 0.076bc	6.40 ± 0.058bc	6.20 ± 0.115cde	6.00 ± 0.100efg	5.80 ± 0.000g	**20**
6.000 ± 0.054cd	6.20 ± 0.058cde	6.10 ± 0.058def	5.90 ± 0.058fg	5.80 ± 0.058g	**30**
5.925 ± 0.046d	6.10 ± 0.058def	6.00 ± 0.058efg	5.80 ± 0.058g	5.80 ± 0.058g	**40**
	6.44 ± 0.083a	6.24 ± 0.065b	5.98 ± 0.047c	5.82 ± 0.028d	**Average**

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Oxidative stability assessment through the determination of Thiobarbituric Acid Reactive Substances (TBARS) is an essential criterion for chicken burgers quality, measuring the degree of lipids oxidation caused by the storage process and technological methods used.

The results presented in **Table (5)** for chicken burger samples refrigerated at 4°C Over a 15 day period showed that TBARS values were significantly affected ($P<0.05$) by both ozone exposure duration and storage time. The control sample recorded the lowest oxidation value on day 0 at 0.223 mg MDA/kg, but underwent sharp, significant increases to reach 2.100 mg MDA/kg on day 15, with an overall mean of 1.141 mg MDA/kg for the control.

This accelerated increase in the control sample is consistent with the findings of [5, 4]regarding lipid oxidation during prolonged storage, where continuous exposure to oxygen leads to the accumulation of secondary oxidation products, as further corroborated by the scientific reviews of [38, 26] concerning the role of microbial spoilage in accelerating sensory quality deterioration.

In contrast to the control sample, ozone treatments exhibited a different response. In the 10-minute treatment, an initial value of 0.214 mg MDA/kg was recorded on day 0, which increased to 0.920 mg MDA/kg on day 15, yielding an overall mean of 0.529 mg MDA/kg. With increasing exposure duration, TBARS values rose progressively, 20-minute treatment recorded an overall mean of 0.654 mg MDA/kg (with values ranging from 0.217 mg MDA/kg on day 0 to 1.200 mg MDA/kg on day 15). This upward trend continued in the 30-minute treatment, which exhibited an overall mean of 0.778 mg MDA/kg (progressing from 0.221 mg MDA/kg to 1.420 mg MDA/kg).

As for the 40-minute treatment, it recorded the highest values among the ozone-treated samples, with an overall mean of 0.921 mg MDA/kg (with values ranging from 0.223 mg MDA/kg on day 0 to 1.680 mg MDA/kg on day 15).

Table 5: Effect of ozone exposure durations (10, 20, 30, and 40 min) on Thiobarbituric acid reactive substances (TBARS) values (mg MDA/kg) in chicken burger samples stored at 4°C for 15 days.

Average	Storage Time at 4°C (days)				Ozone Exposure Time (min)
	15	10	5	0	
1.141 ± 0.210a	2.100 ± 0.021a	1.420 ± 0.020c	0.820 ± 0.015g	0.223± 0.007n	**0**
0.529 ± 0.080e	0.920 ± 0.012f	0.600 ± 0.006j	0.380 ± 0.007m	0.214 ± 0.003n	**10**
0.654 ± 0.111d	1.200 ± 0.015d	0.750 ± 0.021h	0.450 ± 0.010l	0.217 ± 0.006n	**20**
0.778 ± 0.134c	1.420 ± 0.020c	0.920 ± 0.026f	0.550 ± 0.010k	0.221 ± 0.007n	**30**

ISSN 2072-3857

0.921 ± 0.162b	1.680 ± 0.021b	1.100± 0.010e	0.680 ± 0.010i	0.223 ± 0.009n	**40**
	1.464 ± 0.108a	0.958± 0.077b	0.576 ± 0.042c	0.220 ± 0.003d	**Average**

It can be observed that there is a reverse relationship in the statistical correlation of spoilage indicators, meaning while the control sample possessed the highest counts of total aerobic, lipolytic and proteolytic bacteria, the increase in these microbes was associated with the drastic raise in pH values due to the accumulation of basic by-products such as ammonia and amines [23, 26]. However, the application of ozone helped stabilize pH values in the normal range and reduced the enzymatic activity of microbes as reflected through the increase in the secondary product of breakdown TBARS.[20]

It is important to note that this seeming contradiction between the stability of pH values and decrease in microbial load, on the one side, and the slight increase in TBARS values, on the other side, supports the opinion of [54] that ozone does not only act as a surface sanitizer, but as a chemical modification technique that significantly reduces the activity of microbial protease and lipase enzymes [22] in order to provide the stability of biochemical and chemical properties of meat. These results correlate with the findings of [21], because ozone is an oxidizing gas that attacks the unsaturated fatty acids of chicken tissue and causes lipid oxidation as the secondary effect along with its sanitizing effect. Although the results obtained in Table (5) contradict the control sample in terms of TBARS which is more susceptible to the oxidation caused by the action of microbes, they are consistent with the comprehensive reviews conducted by [47, 20] about the induction of oxidative stress caused by the emerging technologies. Comparing the daily means, the overall mean value on day 0 was 0.220 mg MDA/kg, then it was raised to 0.576 mg MDA/kg on day 5, then to 0.958 mg MDA/kg on day 10, and finally to 1.464 mg MDA/kg on day 15, and this signifies the significant increasing trend associated with the period of storage.

According to the studies conducted by [22, 7], the choice of the time of exposure to ozone should combine two opposing factors – the reduction of the microbial load and the preservation of the oxidative stability of meat. Thus, the treatments that exceed 30 – 40 min have better efficacy in reducing bacterial counts, but they leave the oxidative mark that might influence negatively the flavor, and therefore, they should be used in strictly controlled systems.

Statistical data obtained in the course of the current study confirm that the effect of ozone treatment consistently stayed within the higher safety margin in comparison with the control sample, so it can be considered as the promising technological approach provided that the duration of exposure is controlled in order not to exceed the sensory rancidity threshold.

The research estimated sensory attributes including general appearance, taste, flavor, juiciness, tenderness, and overall acceptability using the 9-point Hedonic Scale, as well as the off-odor intensity using the Intensity Scale. Such choice of the methodological approach is justified scientifically by the principles defined by [29], because the hedonic scale is recognized as the most precise tool for

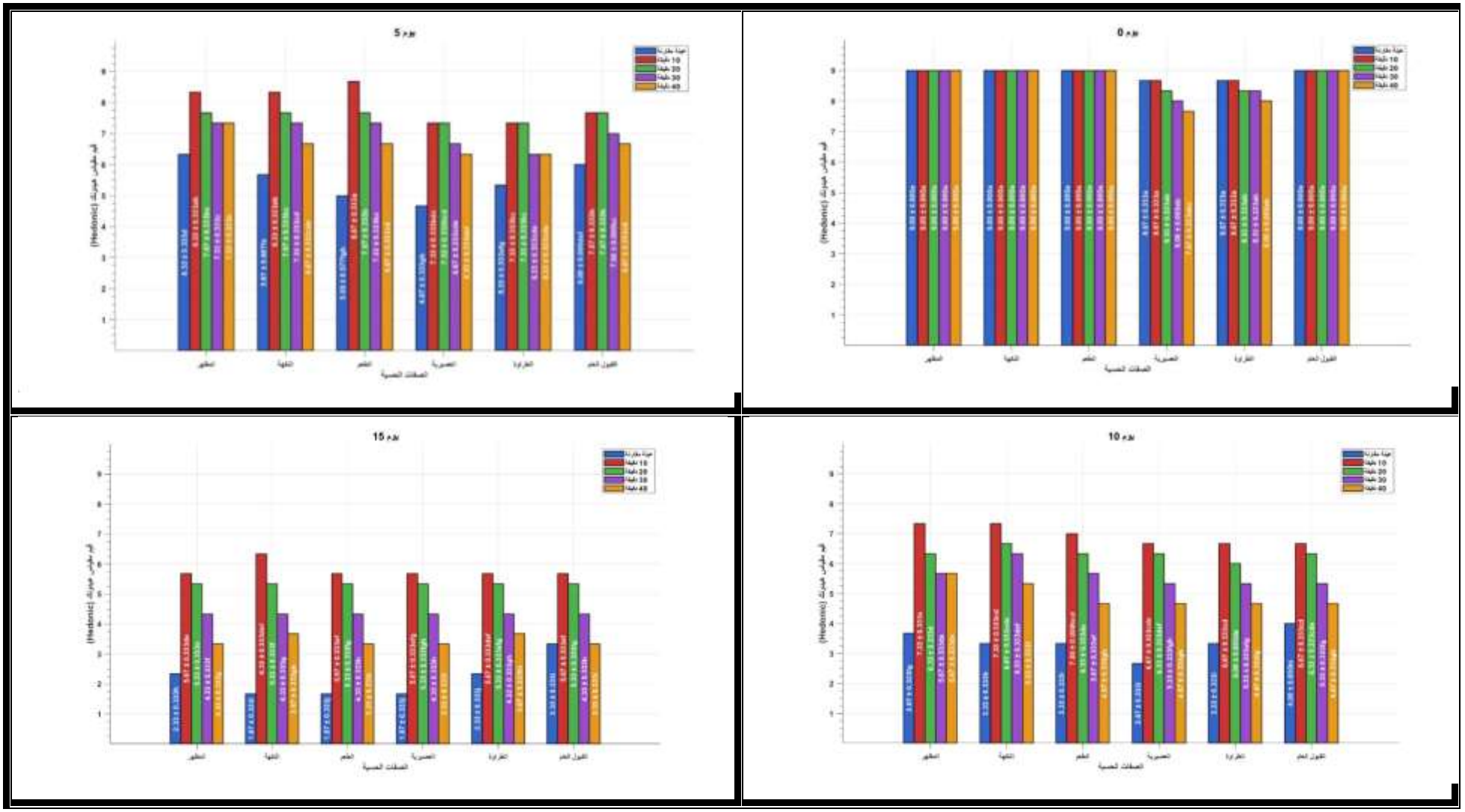
measurement of consumer liking and acceptability, and the intensity scale helps in identifying the deterioration of sensory quality. Radar chart was used for the visualization of sensory evaluation according to the approach suggested by .[27]

As it can be seen in Figure (1) showing the sensory evaluation results for the samples of chicken burger stored at 4°C, there were significant differences ($P < 0.05$) among the treatments during the 15 days of storage period. As for the control sample, there was a sharp drop in the scores of all sensory attributes: the appearance scores decreased from 9.00 to 2.33, flavor from 9.00 to 1.67, taste from 9.00 to 1.67, juiciness from 8.67 to 1.67, tenderness from 8.67 to 2.33, and the overall acceptability was 3.33. However, there was significant superiority of the moderate treatments (10 and 20 min), because the 10-minute treatment maintained higher scores at day 15 for appearance (9.00 to 5.67), flavor (9.00 to 6.33), taste (9.00 to 5.67), juiciness (8.67 to 5.67), tenderness (8.67 to 5.67), and overall acceptability (9.00 to 5.67), as well as the 20-minute treatment scored appearance (9.00 to 5.33), flavor (9.00 to 5.33), taste (9.00 to 5.33), juiciness (8.33 to 5.33), tenderness (8.33 to 5.33), and overall acceptability (9.00 to 5.33). On the contrary, the intensive treatments (30 and 40 min) experienced the significant reduction, and the overall acceptability scores became 4.33 and 3.33 at day 15, respectively.

This relative stability observed in moderate treatments is attributed to the inhibitory properties of ozone against microbial load, which the results demonstrated to increase with prolonged storage duration and decrease with extended exposure time, in agreement with the discussion by [43]regarding the role of modern technologies in enhancing meat safety and quality.

Chemically, TBARS values were observed to increase with prolonged exposure duration and storage time, explaining the sensory modifications in flavor, [52] noted that excessive oxidation in ozone-treated products accelerates the formation of rancid off-flavors. Furthermore, the elevation in pH with increasing storage time and exposure duration is driven by the activity of Proteolytic bacteria that release basic nitrogenous compounds.

ISSN 2072-3857



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Figure 1: Effect of ozone exposure durations (10, 20, 30, and 40 min) on sensory attribute scores (appearance, flavor, taste, juiciness, tenderness, and overall acceptability) of chicken burger samples stored at 4°C for 15 days.

ISSN 2072-3857

Regarding off-odor intensity, as illustrated in **Figure (2)**, values in the control sample increased from 1 on day 0 to 8.67 on day 15. In contrast, in the 10-minute treatment, it rose from 1 to 3.67, and in the 20-minute treatment from 1 to 4.33, whereas intensive treatments (30 and 40 min) increased from 1 to 5.33 and 6.33, respectively. This progression is attributed to excessive microbial activity in the control sample, which generates sulfurous compounds and ammonia, consistent with the findings of [41] regarding the efficacy of processing techniques in controlling undesirable off-odors.

The stabilization of off-odor intensity in moderate exposure durations (10 and 20 min) is attributed to the balanced efficacy of ozone in controlling microbial proliferation without inducing acute lipid oxidation that elevates TBARS values, as observed in intensive exposure durations.

Thus, it is scientifically established that the moderate durations (10 and 20 min) represent the optimal treatments, successfully balancing microbial preservation with the retention of sensory quality in chicken burger , in contrast to the other treatments that failed to reconcile microbial control with acceptable sensory and physicochemical properties.

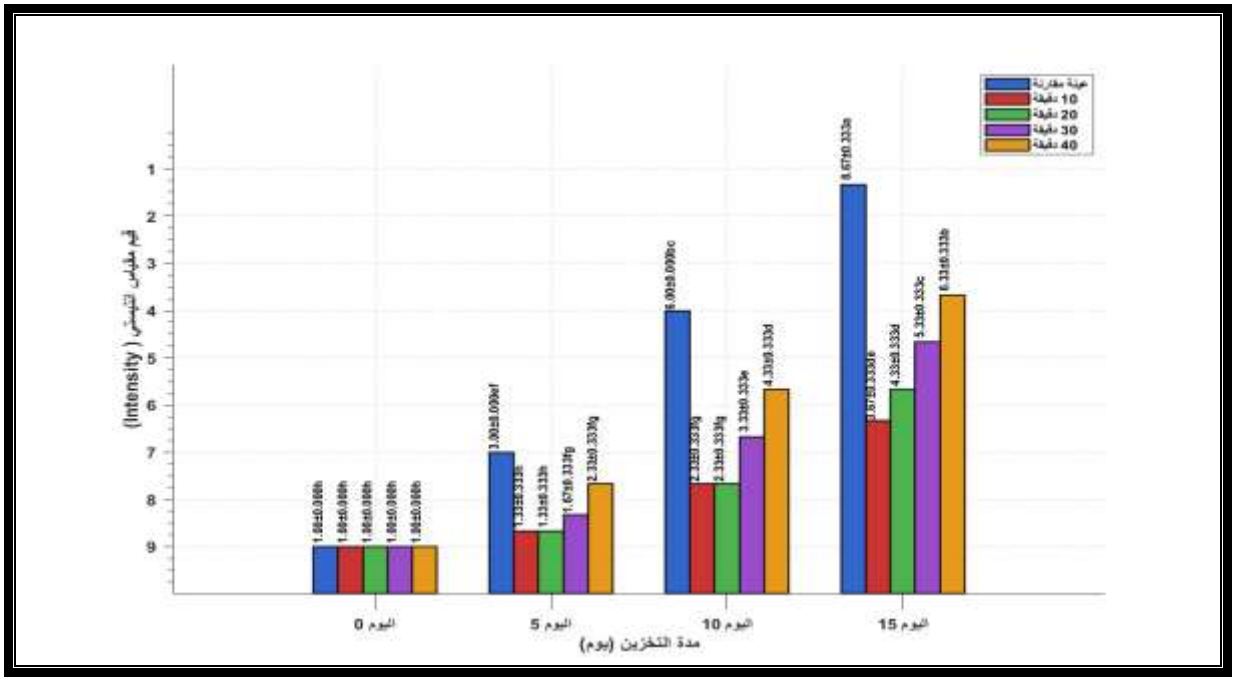


Figure 2: Effect of ozone exposure durations (10, 20, 30, and 40 min) on sensory attribute scores (off-odor intensity) of chicken burger samples stored at 4°C for 15 days.

Conclusion

The experiment showed that the use of aqueous ozone (600 ppm) was an efficient, non-residue preservation technique that increased the microbial safety and refrigerated shelf-life of chicken burger at 4°C. Although long contact times (30 and 40 minutes) maximized microbial reduction and pH control, they caused serious oxidation of lipids, hence higher

ISSN 2072-3857

levels of TBARS and deteriorated sensory characteristics especially flavor and odor.

Conversely, moderate exposure durations of 10 and 20 min established the optimal equilibrium between microbial safety and quality preservation. These treatments significantly suppressed the proliferation of aerobic, proteolytic, and lipolytic bacteria, maintained chemical stability, and kept lipid oxidation well within acceptable limits without compromising sensory attributes such as appearance, tenderness, and overall acceptability throughout the 15-day storage period. Consequently, moderate aqueous ozone application presents a commercially viable, sustainable intervention for the poultry processing industry to extend product shelf life without relying on synthetic chemical additives.

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Conflict of interest

The authors declare that they have no conflict of interest relating to the publication of this research work.

Author contributions

YA-A 33.3%, OHA-J 33.3%, ASR 33.3% – study conception and design.

YA-A 33.3%, OHA-J 33.3%, ASR 33.3% – acquisition of data.

YA-A 33.3%, OHA-J 33.3%, ASR 33.3% – analysis interpretation, writing, and approval of the final manuscript.

This research paper is extracted from the Ph.D. dissertation of the first author.

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