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RESEARCH ARTICLE OPEN ACCESS

Studying the Penetration Ability of Various Pathogenic Bacteria into Raw Beef Meat Surface and the Antibacterial Effect of Ozonated Water

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Abstract

Colonization of meat with bacterial pathogens potentially affects the quality of the meat products. This study aimed to determine the penetration ability of psychrotolerant bacterial pathogens into the surface of red beef meat. The penetration experiment was carried out on raw beef meat to see how different time intervals (1 day, 2 days, and 3 days) and temperatures (5°C, 15°C, and 20°C) affected the penetration process. When bacteria are found at a depth of about 2 cm in the tested meat block, this is considered a positive penetration occurrence. In this study, the action of ozone treatment (at variable exposition times) on the bacterial loads of beef meat samples in which bacterial penetration occurred was evaluated. The formation of ozone was accomplished by utilizing an O_3 generator (A2Z/AQUA-6, USA). The O_3(ppm) concentration in water was defined using the HI 38054 Ozone Test Kit (Hanna Instrument®, USA). The results of bacterial penetrability suggest that at low temperatures, the rate of germ penetration onto the meat surface decreases. The results revealed that the penetration rate increased in the case of dual and triple bacterial species. This can be explained by the fact that all the bacterial species that were used in the study are actively motile and have proteolytic activity. These proprieties together absolutely play a major role in the acceleration of the penetration process by these bacteria, or even so, their existence together is synergistic for migration deeper into meat. To ensure freshness and public health protection, raw meat must be treated and processed in a sanitary manner. Under the conditions used in this study, a concentration of 0.5 ppm of O_3 was found to be highly effective in reducing the bacterial count in meat blocks. This decline was aided by rising the exposing time to 45 minutes to achieve 3 log10 CFU/ml^{-1}, and the bacterial count diminished with increased exposure time to ozonated water at the same concentration. These results suggest that O_3 treatment could be used to lay the groundwork for a new method of reducing meat contamination by foodborne bacterial pathogens.

Keywords: Bacteria, Penetration, Beef meat, Antibacterial effect, Ozonated water

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INTRODUCTION

Beef consumption is projected to increase (FAO).[1] Meat surfaces tend to be susceptible to colonization by a variable of microorganisms that exist in the processing environment.[2] The presence of bacteria on meat surfaces has been identified as the first stage in bacterial contamination of freshly slaughtered meat products. Colonization of meat with bacterial pathogens has a potential effect on the quality of the meat product.[3] The initial attachment of bacteria to raw meat surfaces is unclearly understood, leading to a wide range of bacteria, some of which could be pathogenic. The occurrence of such bacteria on meat surfaces can lead to an outbreak of diseases. The prime sources of bacterial contamination of meat are the animal sources of bacteria, the plant workers, and the processing environment.[4]

Bacterial pathogens, mainly those that originate from animals, can be predicted to be found occasionally dominating the primary microflora and this is significant for public health. The microbiological features of meat-borne pathogens that cause food poisoning vary in accordance with the nature of the bacterial pathogen.[5] *Aeromonas hydrophila, Listeria monocytogenes,* and *Yersinia enterocolitica* are listed among the microorganisms that cause food poisoning related to raw meat. Besides being psychrotolerant, their mode of pathogenicity tends to cause infection rather than intoxication.[5] Rolling a sterile cotton swab over the surface of raw meat to eliminate germs is one technique for assessing the degree of bacterial contamination. Bacterial contamination, according to this method, may only occur on the surface of the flesh and not in the core, deeper layers.[6] During the logarithmic phase of growth development, after reaching their maximum cell density, the bacteria remain on the top surface of the meat, producing extracellular protease enzymes that appear to cause damage, and the bacteria are able to penetrate the deep layers of meat tissue by facilitating connective tissue between muscle fibers.[7]

Bacteria have been reported to infiltrate through the surface of the meat, resulting in food deterioration and consumer food poisoning. Recognizing the process of bacterial penetration into meat enables the construction of stronger meat microbial protection mitigation measures (better food contamination prevention and inspection).[8] An improved perception of the ability of bacterial pathogens to penetrate in the process of penetration might be helpful in devising approaches for reducing public health risks. However, investigations into bacterial pathogens' penetration into raw meat surfaces are scarce but crucial in order to understand the underlying nature of contamination. Meat products' microbiological safety threats, as well as the target of shelf-life extension, necessitated strategies that work. In the last few years, various new technologies developed for increasing food safety have been created owing to the distasteful predicament caused by the thermal treatments on several food products, for instance, the decline in both physical and nutritional values of the food. Gaseous ozone is one of the recent non-thermal methods that are pragmatic to preserve food products safely and at their best quality.[8]

Ozone is a disinfectant that is also a safe oxidant. Today, the use of ozone (in a gaseous phase) has several advantages. One of the most interesting features is that excess ozone can easily decompose to create oxygen without leaving traces in foods.[9] The numerous advantages of ozone make it one of the few best candidate techniques attracting the attention for the food industry. Ozone is among the most powerful sanitizers known. This sanitizer is effective against various types of germs at quite low concentrations.[10] Ozone is an antibacterial agent that is effective against a number of foodborne pathogens and has greater antimicrobial efficacy than other antimicrobial agents.[11] Different countries have employed the ozonation treatment procedure. Ozone's bactericidal action has been verified on a wide range of species, including gram positive and gram negative bacteria, bacterial spores and vegetative cells, fungi, molds, and viruses.[11] The Food and Drug Administration of the United States (FDA) certified ozone as GRAS (Generally Recognized As Safe) to be used in food safety in 1997, and the FDA released its final decision in 2001, approving the rules and guidelines for its usage.[12] Ozonation methods with appropriate ozone concentration and exposure time have been legally approved in food processing in many

European countries, such as North America, Australia, and Japan.[13] The use of ozone in beef meat will successfully prevent microbial growth. Nevertheless, the effects of ozone on meat color and rancidity must be researched. Since ozonation demands specialist equipment, the treatment's efficiency in terms of microbial load reduction and meat quality features must be examined in order to justify the equipment expense.

The following were the aims of this study: -to test the penetration ability of *Aeromonas hydrophila, Listeria monocytogenes* and *Yersinia enterocolitica* (isolates from food of animal sources) into the surface of red beef meat and to verify whether time and temperature impact the penetration process. To compare the penetration ability results obtained by using single bacterium species, dual bacterial species, and triple bacterial species, respectively and to investigate the impact of ozone treatment on the bacterial burdens of beef meat samples that showed bacterial penetration (at varied exposure durations).

MATERIALS AND METHODS

Bacterial isolates growth conditions: three species of psychrotrophic bacteria were selected to check the ability of those bacterial species to penetrate the surface of beef meat. To enable comparability, strains were identified to represent both gram-positive and gram-negative foodborne hazards. The source of these bacteria was isolated from food sources such as animal sources sold at local markets in Baghdad. The bacterial species were: *Aeromonas hydrophilia, Listeria monocytogene* and *Yersinia enterocolitica.* The selected bacteria strains of the study were diagnosed to carry the genes for virulence factors that may cause human infection, which were confirmed by conventional PCR in which *Y. enterocolitica* carried both (Yst and YAdA genes) in the case of *Listeria monocytogene* carried prfA while in *Aeromonas hydrophilia* (aer) and (ast).

The bacteriology stock culture was collected from the public health (food hygiene) department of the college of veterinary medicine at Baghdad University's bacteriology stock. Frozen stockpiles of strains were stored, which were plated on to the tryptic soy agar and cultured for 24 hrs at 37°C to yield individual colonies before stockpiling at 4°C. Also, the motility of each bacterial species was checked by motility tests by the formation of the formazan structure on the motility tests, which were applied on semisolid medium with TTC stain. The isolates' proteolytic activity, which was seen by the hydrolysis of casein on agar plates supplied with 0.5 percent casein, 0.5 percent glucose, and two percent agar at pH 7.0, validated the isolates' proteolytic activity.[14] After the incubation of the plates at 28°C for 7-8 days, the enzyme activity was characterized by the establishment of a clear zone around the colonies after addition of the 1 M HCl solution. A commercial protease solution (Sigma P-4032) at 0.001 percent (w v -1) was used as the positive control.

Preparation of bacterial culture inoculum

All cultures were maintained on stock cultures of each chosen bacterial isolate, and bacterial inoculum for all study experiments was prepared by taking a loopful of bacterial culture and sub-culturing it into 10 ml of the double strength trypticase soya broth-yeast extract (TSB-YE) and incubating it at 25°C for 24 hours. After that, decimal dilutions were fitted into TSB. From suitable dilutions, 1 ml of culture suspension was added to obtain about (15×10^8) cells per ml in the culture tube. An equal number of the two types of tubes are created: one with a single type of bacterial species and the other with a cocktail of two or three types of bacterial species that are used to test penetration ability.

Meat block preparation

Meat blocks were cut according to the dimensions of 2 cm x 3 cm x 6 cm. The meat block surface was sterilized with a hot knife. The types of meat blocks used to investigate bacterial penetrability in this study were fat-free beef, a polystyrene petri dish with a thin layer of nutrient agar, and each meat block was inserted into the base of the petri dish and a hole was made with a glass rod, which was then sealed with parafilm. The remaining nutrient agar was poured over the meat blocks until they were fully coated. The not burned surface of the meat blocks was inoculated by the bacterial isolates of the study to provide an initial bacterial count of $15 \times 10^8/cm^2$.

The bacterial penetration occurrence in the meat blocks was tested under three different

temperatures by the incubation. The meat blocks were stored under controlled isothermal conditions at 5°C, 15°C, and 20°C.[15] The penetration occurrence was determined by removing a cross-sectional meat strip 1 cm aseptically using sterile instruments, starting from the end of inoculation at three-time intervals (1 day, 2 days, and 3 days, respectively). In 10 ml of peptone water, the meat slice was homogenized and a complete loop of these homogenates was streaked on agar plates. The penetration was deemed to be positive when the bacteria were located at least 2 cm deep in the meat strip. It is remarkable that each experiment was applied in triplicate.

Calculation of O_3 concentration production (ppm in water)

The HI 38054 Ozone Test Kit (Hanna Instrument® USA) has been used to test the concentration (ppm) of O_3 in water delivered by the O_3 generator (A2Z/AQUA-6 Specifications). The steps below quickly explain how to compute the O3 concentration according to the kit's brochure.

1. One glass vial was filled with 5 ml of sample, and one of them was inserted into the disc checker's left-handed opening as a blank.
2. In the other vial, deionized water was applied up to 10 mL level. For mixing, the cap was replaced and shaken.

Then the cap was removed and 1 packet of HI 93757-0 reagent was added (the appearance of a pink tint in the sample is proportional to the ozone concentration). The cap is replaced and shaken for mixing. Allow 2 minutes for the reaction to take place. The cap was removed, and the reacted sample was inserted into the disc checker's right-hand aperture. Then the checker disc was held such that a light source illuminated the samples from the rear of the windows, and the color matched. When the disc revolved and was staring at the color test windows, it stopped when the color matched. The value is read immediately in the result window in ppm of ozone. The greatest concentration that was recorded throughout the 3 periods utilized was recorded at 15, 30, and 45 minutes, which was at a concentration of 0.5 ppm/ in water.

The influence of ozonated water (0.5 ppm) on beef blocks where bacterial penetration with pathogenic bacteria occurred over time. In a nutshell, a plastic container was loaded with 1 liter of water from the tap and sealed with its lid. The cap was pierced to enable the insertion of a scrolling aeration stone into the bottle. There were three exposure periods (contact times) specified (15, 30 and 45 min) (15, 30, and 45 min). The tap water was replaced with new tap water after each exposure period, and the process was repeated. In this test, by using an aeration stone (diffuser), ozone gas was injected into the water and blown out consistently throughout the water. As a feed gas, the O3 generator received 1 liter per minute (600 mg/h) of compressed air as a feed. The beef blocks were soaked in ozonated water after being coated with gauze. Three different periods were included (15, 30 and 45 min) through which the ozonated water had already been disseminated throughout the meat samples.[16] The bactericidal impact of O3 was accomplished by calculating the number of viable bacterial counts (CFU) in a viable bacterial suspension in which a series of decimal dilutions of enriched broths were diluted by using sterile peptone water tubes (1 ml of broth/1 ml of peptone water) followed by serial dilutions, and then, a direct drop spreading plating of 20 µ (five drops) of diluted bacterial growth was dropped onto nutrient agar surface and allowed to dry before being incubated at 37°C for 24 h. The bacterial population titer is calculated as follows:

The number of surviving cells ($CFU.ml^{-1}$) was then compared among the different time points.

RESULTS

Bacterial Penetrability to Meat Tissue

The ability of penetration of different pathogenic bacteria in three-time intervals and at three different incubation temperatures is shown in Table 1. *Aeromonas hydrophila* was able to penetrate meat after 3 days in both incubation temperatures (5°C and 15°C). While *Listeria monocytogenes* couldn't achieve penetration to the meat surface at 5°C, penetrability appeared at 15°C after 3 days. In *Yersinia enterocolitica,* the bacterium's penetration ability occurred at 5°C after 3 days and at 15°C after 2 days.

In the case of the experiment in which dual species of bacteria were tested for penetration ability, the bacterial species showed the ability to penetrate the meat surface after 3 days at a 5°C incubation temperature. At 15°C, the dual bacterial

Table 1. Penetration ability of different bacteria into meat surface at three different incubation temperatures

Bacterial species at initial count 9.3 (log CFU*.ml^{-1}) that artificially induced on the surface of meat blocks	Time required for penetration occurrence of bacterial species at depth of 2 cm of beef meat block at different incubation time intervals after /days			
	After 1 day	After 2 days	After 3 days	Incubation Temp.
Aeromonas hydrophila	-*	-	+*	5°C
	-	-	+	15°C
	-	+	+	20°C
Listeria monocytogenes	-	-	-	5°C
	-	-	+	15°C
	-	+	+	20°C
Yersinia enterocolitica	-	-	+	5°C
	-	+	+	15°C
	-	+	+	20°C

+*=refer to existence of penetration at 2 cm depth.,-*= refer to no penetration existence, CFU=Colony-Forming Units.

Table 2. Penetration ability of dual bacterial species into meat surface at 5°C

Dual Bacterial species atinitial count 9.3 (log CFU.ml^{-1}) that artificially induced to one surface of meat blocks	Time required for penetration occurrence of bacterial species at depth of 2 cm of beef meat block at different incubation time intervals after /days.		
	Incubation Temperature = At 5°C		
	After 1 day	After 2 days	After 3 days
Aeromonas hydrophila and *Yersinia enterocolitica*	-	-	+
Listeria monocytogenes and *Yersinia enterocolitica*	-	-	+
Aeromonas hydrophila and *Listeria monocytogenes*	-	-	+

Table 3. Penetration ability of dual bacterial species into meat surface at 15°C

Dual Bacterial species at initial count 9.3 (log CFU.ml^{-1}) that artificially induced to one surface of meat blocks	Time required for penetration occurrence of bacterial species at depth of 2 cm of beef meat block at different incubation time intervals after /days		
	Incubation Temperature = At 15 °C		
	After 1 day	After 2 days	After 3 days
Aeromonas hydrophila and *Yersinia enterocolitica*	-	-	+
Listeria monocytogenes and *Yersinia enterocolitica*	-	+	+
Aeromonas hydrophila and *Listeria monocytogenes*	-	+	+

species *Aeromonas hydrophila* and *Yersinia enterocolitica* show penetration after three days. The other dual species of bacterium could penetrate after 2 days at the same incubation temperature, at 15°C. All the dual bacterium species that were used in the experiment could penetrate the meat surfaces after 2 days at a 20°C temperature. As explained in Tables 2, 3 and 4, also, from the results of this experiment, it seems that the penetration rate of the bacteria to the meat surface decreases at the low temperature.

In the experiment, as explained in table 5 in which used a cockatiel of the triple bacterial species that were tested in the study,

Table 4. Penetration ability of dual bacterial species into meat surface at 20°C

Dual Bacterial species at initial count 9.3 (log CFU.ml^{-1}) that artificially induced to one surface of meat blocks	Time required for penetration occurrence of bacterial species at depth of 2 cm of beef meat blocks at different incubation time		
	Incubation Temperature = At 20°C		
	After 1 day	After 2 days	After 3 days
Aeromonas hydrophila and *Yersinia enterocolitica*	-	+	+
Listeria monocytogenes and *inia enterocolitica*	-	+	+
Aeromonas hydrophila and *Listeria monocytogenes*	-	+	+

Table 5. Penetration ability of cockatiel bacterial species (three bacterial species into meat surface at three different incubation temperatures)

A cockatiel of triple Bacterial species at initial 9.3 (log CFU.ml^{-1}) that artificially induced to surface surface of meat blocks	Time required for penetration occurrence of bacterial species at depth of 2 cm of beef meat blocks at different one one incubation time intervals after days			
	After 1 day	After 2 days	After 3 days	Incubation Temp.
Aeromonas hydrophila, Listeria monocytogenes and Yersinia Enterocolitica	-	+	+	5°C
	-	+	+	15°C
	+	+	+	20°C

Table 6. The antibacterial impact of ozonated water (0.5 ppm) on the decrease of bacterial count (Log10 CFU.$^{-1}$)

Initial count of the bacterial load in meat blocks (species cocktail)	Log10 CFU.ML^{-1} count after ozone treatment in the three points times intervals		
	15 minutes	30 minutes	45 minutes
$2x10^{18}$ (18.3 log)	$17x10^{15}$ (16.2 log)	$32x10^{12}$ (13.5 log)	$82x10^{9}$ (10.9 log)

the penetration ability occurred earlier after one day of incubation at 20°C, while penetration to the meat surface occurred after 2 days of incubation at both (5°C and 15°C) as illustrated in table 5.

The result of the ozonated water effect on contaminated meat blocks

The result of applying ozonated water showed that after the meat blocks (contaminated with a cocktail of triple bacterial species) treatment with ozonated water (0.5 ppm/15, 30, 45 minutes), the initial count of the bacteria was dropped by 3 log10 (log CFU.ml^{-1}) and this depletion was made greater with the expansion of the exposure time to O3 water for 30 min, the count level decreased by another 3 log 10 (log CFU.ml^{-1}). At the final time point of 45 minutes, the log count fell by another 3 logs. Therefore, the count was reduced by 3 logs (log cf.ml^{-1}) at each time point that was utilized in the experiment as shown in Table 6.

The effect of ozonated water on meat blocks contaminated with a single bacterial species results revealed that the ozonated water was effective in decreasing the bacterial count in the case of meat blocks contaminated with *Listeria monocytogene*. The initial count in the meat blocks before treatment was $78x10.^{18}$ While after treatment, the bacterial counts declined by about 2 log 10 (log CFU.ml^{-1}) at each time point. In the blocks that were contaminated with the other dual species (*Yersinia enterocolitica* and *Aeromonas*

Table 7. The antibacterial impact of ozonated water (0.5 ppm) on the diminution of bacterial load (Log10 CFU ml^{-1})

Initial count of the bacterial load in meat blocks	Log10 CFU.ml^{-1} count after ozone treatment in the three points times intervals		
	15 minutes	30 minutes	45 minutes
Yersinia enterocolitica $55x10^{15}$ (16.7 log)	$63x10^{12}$ 13.7(log)	$95x10^{9}$ 9.3(log)	$82x10^{5}$ 6.9(log)
Listeria monocytogene $78x10^{18}$ (19.89 log)	$67x10^{16}$ (17.8 log)	$14x10^{14}$ (15.1 log)	$93x10^{12}$ (13.9 log)
Aeromonas hydrophilia $1.5x10^{19}$ (19.17 log)	$97x10^{16}$ (17.9 log)	$2x10^{14}$ (14.3 log)	$9x\ 10^{12}$ (12.9 log)

hydrophilia), the bacterial count was reduced by 3 log10 (log CFU.ml^{-1}) at each time point, as explained in Table 7.

The ozonated water antibacterial activity appeared to be more efficient on gram-negative species than on gram-positive bacteria species. The period of exposure to ozonated water plays a considerable role in enhancing the removal rate of bacterial contamination.

DISCUSSION

While producing meat, contamination occurs as a consequence of human infection via insufficient hygiene measures, as humans may often harbor microorganisms, or as a result of contaminated animals intended for food production.[17] Cross-contamination of meat can also happen as a result of these products' poor hygienic and storage conditions.[18] The mechanism by which bacteria interact with meat surfaces is not completely clear and its influence is influenced by the surrounding environment. This bacterial interaction poses a meat safety or meat spoilage problem. The species of bacterium and the environmental conditions seem to play an important role in the acceleration of meat spoilage.[17] The occurrence of psychrotrophic microbes on meat surfaces is of concern because it not only causes meat spoilage, even when so sorted at a low temperature, but also represents a public health hazard. The purpose of this research was to demonstrate that bacterial contamination of beef meat surfaces can occur and can be spread from the upper surfaces of meat to the deeper layers of meat, and to find out if ozonated water can decrease the level of such bacterial contamination if the meat is treated with it.

The results of the study showed that the pathogenic bacteria could penetrate the meat surface and their ability to produce proteolytic enzymes and be motile definitely play an important role in enabling those bacterial pathogens to penetrate deeper than the upper surfaces of meat. The penetrability of bacterial pathogens is correlated with their proteolytic activity and motility. These two properties enable the pathogen to move freely between muscle fibers and produce proteolytic enzymes that breakdown connective tissue, leading to the contamination of wider and deeper surfaces.[15] Time and temperature seem to affect the rate of bacterial penetration ability. This may be attributable to the bacterial log count increasing with time since the meat represents a nutritional environment for bacterial growth and colonization. The high bacterial log count acts more effectively in producing considerable amounts of proteolytic enzymes that accelerate the penetration process, most likely by hydrolyzing sarcoplasmic proteins in the muscle tissue.[7] Proteolysis reduces the viscosity of sarcoplasmic protein, resulting in increased motility and proteolysis bacteria migrate rapidly.[19]

The results revealed that the rate of penetration was reduced at low temperatures and increased rapidly at temperatures above 5°C. To avoid bacterial growth, one must guarantee that the temperature of food is maintained (held) below 5°C or over 60°C (WHO recommendations). The temperature range of 5°C to 60°C is sometimes referred to as the "danger zone".[20] The kinetics of food degradation reactions are heavily influenced by temperature. The influence of temperature on

the specific growth rate of bacteria is significant since microbial spoilage is a serious problem.[19] Low temperatures operate in the lag phase, which may stretch this to days, months, or even longer than many hours, while stressors and temperature can lengthen this to many days and months. If and when exponential growth occurs, the same stress parameter (s) may restrict cell division rates. To put it another way, cellular stress may frequently extend its lag-phase and lead to lower rates of exponential growth for a particular microbial population, affecting bacterial penetrability.[21] Temperature fluctuations have an instantaneous effect on microorganisms. Another possibility is that species must adjust to the new temperature, resulting in a lag period caused by the stress of the temperature change.[19]

This is to be anticipated biologically, since the cells are out of balance and must change their enzyme pools, for example, to reach a new equilibrium. This finding demonstrates that in both the lag and exponential phases, the bacteria are stressed by a change in temperature. With this information, it is possible to forecast that growth will be slower than usual.[19] The majority of the microbial population of psychrotolerant bacteria can develop at temperatures as low as 0°C but prefer temperatures around 20°C, whereas obligate psychrophiles have optimal temperatures that are higher than the ambient temperature. As a consequence, tiny differences in oceanic temperatures may have a large influence on these microbes' choice of substrates, and hence on the effective size of the nutrient pools they snap up.[22]

The results revealed that the penetration rate increased in the case of dual and triple bacterial species. This can be explained by the fact that all the bacterial species that were used in the study are psychrotrophic, actively motile and have proteolytic activity. These proprieties together absolutely play a major role in the acceleration of the penetration process by these bacteria, or even so, their existence together is synergistic for migration deeper into meat. The effect of ozonated water on reducing the bacterial count in meat blocks was important; this reduction improved when the exposure period was increased to 45 minutes, resulting in a reduction of 3 log10 CFU. ml^{-1}. In terms of public health, this is extremely important. Ozone is extensively employed in a broad variety of items from agriculture, including vegetables, fruits, and fish. It has a strong oxidation potential and functions as a bactericidal and virucidal agent.[16]

The present study's results (Table 7) demonstrated that after three intervals of ozonated water treatment, the bacterial burden of the meat block samples was lowered. Furthermore, following treatment with ozonated water, the findings revealed that the gram negative bacterial levels had been reduced by 3 log10 CFU ml^{-1} at each time period, but the reduction within gram positive bacteria was 2 log10 CFU ml^{-1}, the exposure time was increased to 45 min to achieve a 5 log10 CFU.ml^{-1} decline in bacterial count as compared to the initial count, From the viewpoint of public health, this decrease is important because O_3 is an antibacterial agent with a wide spectrum of action against bacteria, viruses, fungus, protozoa, and both bacterial and fungal spores.[10] The oxidizing potency of free radicals and their capacity to move across biological membranes, damaging cellular components, interrupting normal cellular function, and causing microbiological harm, is assumed to be the cause of ozone reactivity.[16]

Ozone's powerful oxidizing capacity may cause significant damage to bacteria's cell walls and cytoplasmic membranes. Ozone may harm cell structures, including glycolipids, amino acids, and glycoproteins, as well as alter or impede the cell's enzymatic system.[8] These alterations enhanced membrane permeability, leading the cell system to cease operating and microbes to flourish normally.[12] Ozone's impact on bacterial cells primarily results in permanent damage to the fatty acids in the cell membrane, as well as cellular protein deterioration due to oxidation.[23] Ozone (O_3) is a non-radical product of reactive oxygen species (ROS) that is largely responsible for the establishment of food oxidation processes, particularly in lipids, where it destroys essential fatty acids.[20] The solubility of ozone in water and the stability of its interactions with organic and inorganic molecules might impact its antibacterial activity. The antibacterial efficacy of ozone is determined by relative humidity, temperature, pH, and the quantity of organic substances contained in food that may surround and protect cells.[24]

The CFU from treated samples had a smaller number of persisting bacterial cells than untreated samples. According to the findings of this study, the initial bacterial load count should be higher than the initial bacterial load count. Furthermore, the number of live bacteria dropped as the exposure time period to ozonated water was extended at the same level of concentration (0.5 ppm) (0.5 ppm). Norasak et al.[25] observed that ozonated water spray chill decreased *E. coli* O157: H7 by 0.60 log, whereas aqueous ozone spray chill dropped *E. coli* O157: H7 by 1.46 log on raw beef surfaces, proving the effectiveness of O_3 therapy during immersion cooling as an intervention to increase the microbiological protection of beef meat.

The results of this investigation are congruent with those of Cho et al.[23] who reported that ozone exposure constantly eradicated *E. coli* O157: H7 from the surface of beef samples and that it might be employed as an antibacterial treatment for meat products as the pathogen's initial contamination in meat products was reduced. In the scientific literature, a wide range of findings have been published, depending on the ozone concentrations used and how they were applied.[23]

Several studies have looked into whether using ozone in beef will help to reduce microbial growth (Gimenez et al.)[12] studied the impact of ozone on microbial load in beef meat and discovered that ozone is one of the most efficient therapies for suppressing microbial flora. These treatments allowed for a reduction in the counts of natural flora in beef (lactic acid bacteria, mesophilic, and *Enterobacteriaceae*) by more than a single logarithmic interval. In refrigerated conditions, the counts of injected Listeria monocytogenes (10^2 CFU/g tissue) decreased to levels less than the detection limit. Coll Cárdenas et al. investigated the effect of gaseous ozone treatment on bacterial counts in culture media and meat specimens (total aerobic mesophilic heterotrophic bacterial pathogens and inoculated *Escherichia coli*). The counts of total bacterial aerobic mesophilic heterotrophic microorganisms were reduced by 0.5 log10 cycles, as well as the counts of *E. coli*, reaching 0 log.[26] Ozone has antibacterial properties against viruses as well as gram-positive and gram-negative bacteria.[27] In the previous study (Khudhir and Mahdi),[28] the results indicated that the hurdle method, as ozonated water and storage temperature, can act synergistically to minimize the total bacterial counts, coliforms, yeasts, and mold populations of the local bovine and ovine soft cheese in Baghdad city. The diminished bacterial population was the most significant for ozonated water.

In general, most studies that used ozone to control bacterial contamination in meat found that ozone's antimicrobial activity primarily affects and acts on the surface area of ozonated meat, with a percentage of bacterial load reduction approaching 99 percent.

CONCLUSION

In this investigation, we attempted to investigate the antibacterial impact of ozone on the deeper surfaces of contaminated meat, and the ozone was efficient in reducing the microbial load, but not the bacterial load to an acceptable level.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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None.

DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

This study was approved by the Medical Research Bioethical Committee of the College of Medicine, University of Kerbala, Iraq with reference number 26.

REFERENCES

1. OECD/FAO (2020). OECD-FAO FAO Agricultural Outlook. 2020-2029. OECD Publishing, Paris. 2020. doi: 10.1787/1112c23b-en
2. Heir E, Moretro T, Simensen A, Langsrud S. *Listeria monocytogenes* strains show large variations in competitive growth in mixed culture biofilms and suspensions with bacteria from food processing environments. *Int J Food Microbiol.* 2018;275:46-55. doi: 10.1016/j.ijfoodmicro.2018.03.026
3. Giaouris E, Heir E, Hebraud M, et al. Attachment and biofilm formation by foodborne bacteria in meat processing environments: causes, implications, role of bacterial interactions and control by alternative novel methods. *Meat Sci.* 2014;97(3):298-309.doi: 10.1016/j.meatsci.2013.05.023
4. Pellissery AJ, Vinayamohan PG, Amalaradjou MAR, Venkitanarayanan K. Spoilage bacteria and meat quality. *Meat Quality Analysis.* 2020;307-334. doi: 10.1016/B978-0-12-819233-7.00017-3
5. Bintsis T. Foodborne pathogens. *AIMS Microbiology.* 2017;3(3):529-563.doi: 10.3934/microbiol.2017.3.529
6. Nagarajan V, Wahab A, Shivraj S, Alex L. Study of Bacterial Contamination of Raw Meat in Hyderabad. *MOJ Proteomics & Bioinformatics.* 2018;7(1):46-51. doi: 10.15406/mojpb.2018.07.00212
7. Shirai H, Datta AK, Oshita S. Penetration of aerobic bacteria into meat: A mechanistic understanding. *Journal of Food Engineering.* 2017;196:193-207. doi: 10.1016/j.jfoodeng.2016.10.012
8. Sheng L, Hanrahan I, Sun X, Taylor MH, Mendoza M, Zhua M-J. Survival of *Listeria innocua* on Fuji apples under commercial cold storage with or without low dose continuous ozone gaseous. *Food Microbiol.* 2018;76:21-28. doi: 10.1016/j.fm.2018.04.006
9. Vithu P, Barma RD, Ranjit S, Aditya M. Ozone technology in food processing: a review. *Trends in Biosciences.* 2015;8(16):4031-4047.
10. Khadre MA, Yousef AE, Kim JG. Microbiological aspects of ozone applications in food: A review. *J Food Sci.* 2001;66(9):1242-52. doi: 10.1111/j.1365-2621.2001.tb15196.x
11. Priyanka BS, Rastogi KN, Tiwari BK. Opportunities and challenges in the application of ozone in food processing. Emerging Technologies for Food Processing. 2nd Ed. 2014;19:335-358. doi: 10.1016/B978-0-12-411479-1.00019-X
12. Gimenez B, Graiver N, Giannuzzi L, Zaritzky N. Treatment of beef with gaseous ozone: Physicochemical aspects and antimicrobial effects on heterotrophic microflora and *Listeria monocytogenes. Food Control.* 2021;121:1-9. doi: 10.1016/j.foodcont.2020.107602
13. O'Donnell C, Tiwari BK, Cullen PJ, Rice RG. Use and generation of ozone as a disinfectant of dairy animal tissues, dairy equipment, and infrastructure. 2013. Patent No. US 8609120 B2. Oxford: Wiley- Blackwell. Heacox D
14. Larsen MD, Kristiansen KR, Hansen TK. Characterization of the proteolytic activity of starter cultures of *Penicillium roqueforti* for production of blue veined cheeses. *Int J Food Microbiol.* 1998;43(3):215-221. doi: 10.1016/S0168-1605(98)00114-7
15. Muhammad MS, Erkihun A, Arshad M, Al-Sultan I. The penetrability of selected bacteria on the raw bovine meat. *J Adv Med Res.* 2012;2:12-17.
16. Kanaan MHG. Antibacterial effect of ozonated water against methicillin-resistant *Staphylococcus aureus* contaminating chicken meat in Wasit Province, Iraq. *Veterinary World.* 2018;11(10):1445-1453. doi: 10.14202/vetworld.2018.1445-1453
17. Abebe E, Gugsa G, Ahmed M. Review Article Review on Major Food-Borne Zoonotic Bacterial Pathogens. *J Trop Med.* 2020;2020:4674235. doi: 10.1155/2020/4674235
18. Sutmoller P, Animal PD, Consultant H. Contaminated food of animal origin : hazards and risk management Prevention. *Sci Tech Rev.* 1997;16(2):1-27. https://www.adiveter.com/ftp_public/articulo324.pdf
19. Zwietering MH, De Wit JC, Cuppers HGAM, Van't Riet K. Modeling of bacterial growth with shifts in temperature. *Appl Environ Microbiol.* 1994;60(1):204-213. doi: 10.1128/aem.60.1.204-213.1994
20. Choe E, Min DB. Mechanisms of antioxidants in the oxidation of foods. *Compr Rev Food Sci Food Saf*. 2009;8(4):345-358. doi: 10.1111/j.1541-4337.2009.00085.x
21. Hamill PG, Stevenson A, McMullan PE, et al. Microbial lag phase can be indicative of, or independent from, cellular stress. *Sci Rep.* 2020;10(1):1-20. doi: 10.1038/s41598-020-62552-4
22. Nedwell DB. Effect of low temperature on microbial growth: Lowered affinity for substrates limits growth at low temperature. *FEMS Microbiol Ecol.* 1999;30(2):101-111. doi: 10.1016/S0168- 6496(99)00030-6
23. Cho Y, Muhlisin JHC, Hahn TW, Lee SK. Effect of gaseous ozone exposure on the bacteria counts and oxidative properties of ground hanwoo beef at refrigeration temperature. *Korean J Food Sci Anim Resour*. 2014;34(4):525-532. doi: 10.5851/kosfa.2014.34.4.525
24. Bahrami A, Baboli ZM, Schimmel K, Jafari SM, Williams L. Efficiency of novel processing technologies for the control of *Listeria monocytogenes* in food products. *Trends in Food Science & Technology.* 2020;96:61-78. doi: 10.1016/j.tifs.2019.12.009
25. Norasak K, Worlie D, Wheeler T. A novel aqueous ozone treatment as a spray chill intervention against *Escherichia coli* O157: H7 on surfaces of fresh beef. *J*

Food Prot. 2019;82(11):1874-1878. doi: 10.4315/0362-028X.JFP-19-093

26. Cardenas CF, Giannuzzi ASL, Zaritzky N. Antimicrobial action and effects on beef quality attributes of a gaseous ozone treatment at refrigeration temperatures. *Food Control.* 2011;22(8):1442-1447. doi: 10.1016/j.foodcont.2011.03.006

27. Remondino M, Valdenassi L. Different uses of ozone: environmental and corporate sustainability. Literature review and case study. *Sustainability.* 2018;10(12):4783. doi: 10.3390/su10124783

28. Khudhir Z, Mahdi AM. The Efficacy of Ozonated Water on the Microbial Quality of Locally Produced Soft Cheese in Baghdad. *Int J Sci Res.* 2017;6(10):338-387. doi: 10.21275/ART20176990