frontiers | Frontiers in Food Science and Technology

TYPE Mini Review
PUBLISHED 06 June 2024
DOI 10.3389/frfst.2024.1351801

OPEN ACCESS

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RECEIVED 07 December 2023
ACCEPTED 16 May 2024
PUBLISHED 06 June 2024

CITATION
Giménez B, Zaritzky N and Graiver N (2024), Ozone treatment of meat and meat products: a review.
Front. Food. Sci. Technol. 4:1351801.
doi: 10.3389/frfst.2024.1351801

Ozone treatment of meat and meat products: a review

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Ozone treatment is a non-thermal method for disinfection; ozone is a powerful oxidizing agent that has been shown to be effective in reducing microbial load, extending the shelf life of meat products. This mini-review covers the analysis of the antimicrobial ozone activity in different meats (beef, poultry, pork, seafood, etc.), emphasizing the ozone application methods (liquid or gaseous phase), the applied concentrations and contact times and the effects of ozone treatment on meat quality, safety, and sensory properties. It has been demonstrated that ozone is effective against a broad range of microorganisms, including both Gram-positive and Gram-negative bacteria, spores, and vegetative cells. The efficacy of ozone depends on various factors, such as concentration, type of treatment, temperature, and presence of organic material. Ozone treatment, known for its rapid decomposition and lack of residue, provides an environmentally friendly alternative to traditional chemical sanitizers. Ozone treatments exhibit promising results in enhancing the safety and extending the shelf life of meat products. According to the findings, the application of ozone is an effective technology for prolonging the shelf life of different types of meats and meat products, requiring careful establishment of conditions on a case-by-case basis.

KEYWORDS

ozone, meats, shelf-life, food safety, meat products, gaseous and aqueous ozone treatments

1 Introduction

Several innovative technologies for food industry to food preservation have been developed over the years, such as high hydrostatic pressure, radiofrequency, high intensity pulsed electric fields, ultrasound, irradiation and ozone treatment. All these technologies have advantages, disadvantages and limitations depending on several factors, such as type of food, temperature, pH, the presence of microorganisms and national regulations.

Interest in ozone has resurfaced in recent times due to consumer demands for less processed foods and fresh safe products, in which the organoleptic and nutritional characteristics are unaltered and there are no chemical residues after treatment (Gimenez et al., 2021; Xue et al., 2023). The application of ozone is a practical technology, economical and green; in food it can be applied in liquid or gaseous form.

Ozone can be generated on-site by several techniques; the most used commercially at the present time are UV radiation, corona discharge, and electrolysis (Prabha et al., 2015; Gimenez et al., 2021; Xue et al., 2023). The on-site production of ozone also eliminates the need for transportation and storage.

Ozone is a powerful oxidant that can be applied in the food industry. It is an allotropic form of oxygen, has stronger antimicrobial activity than chlorine and is considered a broad-spectrum antimicrobial agent that acts against a variety of foodborne pathogens and spoilage organisms (Priyanka et al., 2014). Ozone has proven to be an effective bactericide from Gram positive and Gram negative bacteria, and also inactivates viruses, fungi and degrades mycotoxins on fruits, vegetables, meat, grains and their products (Premjit et al., 2014; Brodowska et al., 2017; Pandiselvam et al., 2018; Niveditha et al., 2021).

Ozone was used as early as the 19th century in water treatment, for the deodorization of industrial waste and washing and disinfection of equipment, for spraying crops, thus avoiding spraying with harmful chemicals, for the elimination of odors in animal housing and for air sterilization.

Excess of ozone auto decomposes rapidly to produce oxygen and it breaks down very rapidly in the presence of food products without any residue (Oner et al., 2011; Pandiselvam et al., 2018; Kulwinder Kaur et al., 2022).

In the United States, ozone has received in 1997 GRAS (Generally Recognized as Safe) classification, and in 2001 the Food and Drug Administration (FDA) officially approved media containing ozone for use in the food industry, also for direct contact with food products, including fish, beef and poultry. (Kim et al., 1999; Gonçalves, 2009).

Ozone inactivates microorganisms due to its high oxidation-reduction potential; it oxidizes the constituent elements of microbial cell walls before penetrating inside the microorganisms; then, ozone also oxidizes essential components such as proteins, enzymes, unsaturated lipids, and nucleic acids; after the cell wall and membrane are damaged, bacterial cells are destroyed (Greene et al., 2012; Brodowska et al., 2017; Pandiselvam et al., 2017).

According to literature, ozone generates a progressive oxidation of vital cellular components in microorganisms. Victorin (1992) identified two mechanisms of microorganism destruction by ozone: a) ozone oxidizes sulfhydryl groups and amino acids of proteins, enzymes and peptides generating shorter peptides; b) ozone oxidizes the double bonds of polyunsaturated fatty acids. The degradation of the unsaturated lipids results in cell lysis. Kim, et al. (1999) reported that in Gram negative bacteria, ozone first attacks the lipoprotein and lipopolysaccharide layers increasing cell permeability and eventually cell lysis. Gram positive bacteria are more resistant than Gram negative bacteria due to the presence of peptidoglycan in the wall (Pandiselvam et al., 2022b). Enterobacteriaceae is a diverse group of bacteria including various human pathogens such as *Salmonella, E. coli, Shigella*, etc.,; the Gram negative bacteria commonly studied is *E. coli* (Khadre, et al., 2001). Ozone interferes with the respiratory system of *E. coli* causing its death (Ingram and Haines, 2009).

The efficacy of ozone microbicidal effect depends on several factors such as ozone concentration, temperature and application methods. Ozone can be applied to food products as a gas or it can be dissolved in water; temperature is one of the most important factors because it affects the stability, reactivity and solubility of the gas (Khadre et al., 2001; Coll Cardenas et al., 2011).

Other factors affecting its performance are: intrinsic properties of food such as aw, pH, additives and the presence of amount of organic matter surrounding the cells (Manousaridis et al., 2005; Priyanka et al., 2014).

Xue et al. (2023), showed a flow chart to summarize ozone effectiveness factors, decontamination mechanisms against bacteria, fungi, mould and biofilms, and the combination of ozone with other preservation technologies (hurdle technology).

Restaino et al. (1995) found that *Listeria monocytogenes* was the most sensitive to ozone among the pathogens studied (*Salmonella typhimurium, Yersinia enterocolitica and Staphylococcus aureus).* Treatment conditions must be determined specifically for each type of product for safe and effective use of ozone.

Another property of ozone is the capacity of absorption of flavors and strange odors in the water, due to the fast destruction of organic compounds; in the same way, ozone has a deodorization role of the air (Gonçalves, 2009).

This mini-review aims to analyze and summarize the influence of ozonation treatments in gaseous and liquid phases applied to different types of meat and meat products. Emphasis is placed on the ozone concentrations utilized in the different treatments and in the units used to express these concentrations in liquid or gaseous phases in order to compare the results reported by the different authors. The review also comprehensively addresses the impact of ozone treatment on the physicochemical characteristics of meat products.

2 Gaseous and aqueous ozone treatments

In food industries, ozone can be applied in gaseous or dissolved in aqueous phase. In order to analyze the information reported in literature, the different ozone concentrations units used by the authors must be considered. Ozone concentrations can be expressed as ppm or mg/L when it is applied in liquid phase. In the case of ozone treatment in gaseous phase, concentrations in air can be expressed by volume or by weight. When volumetric concentrations are used the equivalences are: 1 g O_3/m^3 = 467 ppmv O_3; 1 ppmv O_3 = 2.14 mg O_3/m^3. For concentrations of ozone in air by weight: 100 g O_3/m^3 = 7.8% O_3; 1% O_3 = 12.8 g O_3/m^3; 1% O_3 = 7,284 ppm Ozone.

Ozone is an unstable gas, it cannot be stored, and therefore it must be generated on-site as needed. The methods of ozone generation depend on the concentration requirement. In the UV photochemical method, feed gas (usually ambient air) is passed through the UV lamp (wavelength used is 185 nm) and photo-disassociation splits the oxygen molecules into unstable oxygen radical atoms, which react with oxygen molecules to form ozone; this method produces a low concentration ozone up to 0.3%–0.4% by weight (Cullen and Tiwari, 2012) because the exposure of air to radiation.

The corona discharge method (or plasma) produces higher concentrations of ozone (Cameron and Rice, 2012). In this method, gas (air or dry oxygen) passes through electrodes, which are separated by a dielectric material; as oxygen molecules pass through the medium, they are split into radical atoms (oxygen radicals) with high energy that combines with molecular oxygen to produce ozone (Priyanka et al., 2014). Ozone generation via corona discharge is the most common method that is applied commercially.

After ozone treatment, the surplus ozone should be destroyed due to safety considerations (Brodowska et al., 2017).

Increased concentrations of ozone lead to a more rapid inactivation of microorganisms, resulting in shorter treatment times and smaller Decimal Reduction Time (D) values (Steenstrup and Floros, 2004). It should be noted that higher ozone concentrations may induce the oxidation of certain food compounds (Priyanka et al., 2014).

Ozone is unhealthy for humans who are exposed to this gas at high concentrations even if it is for short periods of time. The toxic properties of ozone may cause specific symptoms, such as drying of the throat, headache, irritation to the nose, possibly severe illness, and even death (Muthukumarappan et al., 2000). Long-term ozone exposure is associated with increased respiratory illnesses, metabolic disorders, nervous system issues, reproductive issues (including reduced male and female fertility and poor birth outcomes), cancer and also increased cardiovascular mortality.

An exposure time of only few minutes at ozone concentrations of 1.0–2.0 ppm produces irritation on the upper part of the throat, headache, chest pain, cough, dry throat; 5.0–10.0 ppm produces increased pulse, edema of lungs, concentrations >50.0 ppm are potentially fatal and concentrations higher than 1700 ppm are lethal (Brodowska et al., 2017).

The Environmental Protection Agency (EPA) established a maximum concentration permitted in air of 0.08 (ppm) for a human exposure time in ozonated air of 8 h (Gonçalves, 2009).

In addition, during ozone generation from oxygen as the feed gas, the workers must pay attention that the flammability of many organic materials can increase dramatically (Brodowska et al., 2017).

In aqueous phase, ozone may be generated by bubbling the gas through water to enable dissolution or via electrolytic methods. In the case of gas bubbling, the ozone solubility in water must be taken into account; this depends on the pressure of water, temperature of water, ionic strength, presence of ionic salts and ozone gas concentration. One of the determinant factors that affect decontamination efficiency is the poor ozone solubility, because it influences on the concentration levels achievable in aqueous solutions (Batagoda et al., 2018; Aslam et al., 2020).

The solubility of ozone in water is ten times higher than oxygen and decreases with an increase of water temperature (Pirani, 2010; Brodowska et al., 2017); ozone is more soluble in water at 0°C (0.6401 ozone/L water) than at higher temperatures. The gas dissolves in water at pH below 7.0; however, an increase in the pH value leads to a spontaneous decomposition of ozone, producing highly reactive free radicals, such as hydroxyl ·OH. At pH = 8, nearly half of the introduced ozone is decomposed to various intermediate forms and to oxygen within 10 min. Ozone decomposes in solution following a stepwise mechanism, producing hydroperoxyl (·HO2), hydroxyl (·OH), and superoxide (·O2 -) radicals (Pirani, 2010). The hydroxyl radical is an important transient species and chain-propagating radical. The reactivity of ozone is attributed to the great oxidizing power of these free radicals. (Pirani, 2010; Brodowska et al., 2017). Ozone causes the formation of free radicals at pH > 8; and at lower pH, the mechanism of ionic reaction predominates (ozonolysis) and generates the peroxide production (Gonçalves, 2009).

In some cases, gaseous ozone offers advantages over aqueous ozone due to its superior penetration capacity, enabling it to reach inaccessible areas in products where pathogens may be present (Shynkaryk et al., 2015).

3 Gaseous ozone treatments

Meat (beef, poultry, pork, seafood, etc.) is widely consumed around the world due to its nutrients for a healthy diet. It is consumed as fresh meat or processed meat. Due to its nutrient richness and high aw, meats are susceptible to microbial attack, thus decreasing their shelf life, being one of the most important sources of foodborne illness (Fearnley et al., 2011; Antunes et al., 2016).

In meat processing there are numerous sources of contamination such as slaughtering and evisceration procedures, improper handling of equipment, poorly sanitized equipment, contaminated washing water and unacceptable temperature conditions (Pandiselvam et al., 2022a).

Ozone disinfection rates, depend on the type of organism and are affected by different factors. Epelle et al. (2023) classified these factors into three categories: environmental conditions, properties related to the substrate/material, and operational properties. These parameters can affect the process, altering the stability of the ozone in the medium (air or water), the efficiency of microbial inactivation, or both.

Table 1 summarizes the effect of gaseous ozone treatment on different microorganisms present in various food matrices (beef, chicken, poultry, seafood). These effects depend on the different ozone concentrations used as well as on their forms of application. In the Table, concentrations were expressed in the units reported by the authors to avoid errors; this is because in many cases the authors used ppm without clarifying if these units are volumetric or by weight.

Coll Cardenas et al. (2011) reported that in beef samples treated with gaseous ozone, the highest microbial inactivation was observed after 1 day at 0°C producing a decrease of 2.0 $\log_{10}$ cycles in total aerobic mesophilic heterotrophic microorganism counts and 0.7 Log cycles in *E. coli* counts. However, these treatments led to unacceptable results of lipid oxidation and surface color. In contrast, exposure times of 3 h at a gaseous ozone concentration of 154 mg/m^3 at 0 or 4°C, reduced only 0.5 log cycles the counts of total aerobic mesophilic heterotrophic microorganisms and 0.6–1.0 log cycles *E. coli* counts, without producing rancidity or changing the color of beef. The use of ozone in conjunction with refrigeration improved CFU reduction, increasing the shelf life of products.

Cho et al. (2014a) studied the effect of ozone in ground Hanwoo beef inoculated with *E. coli.* O157:H7. The treatment consisted of exposing the inoculated samples to 10 mg O_3/h in a chamber of 25 × 20 × 20 cm for 3 days at 4°C; they found that *E. coli* counts were reduced 0.53 log CFU/g after exposure to ozone for 1 day, and bacterial growth was not observed during 3 days of storage.

Lyu et al. (2016) analyzed the combined effect of carbon monoxide (CO) and ozone pretreatment on the quality of vacuum packaged beef. Beef samples were pretreated with gaseous combinations of different volume ratios of carbon monoxide and ozone (100% CO; 2% O_3/98% CO; 5% O_3/95%

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TABLE 1 Effect of gaseous ozone concentration on meats.

Type of meat	Gaseous ozone treatment	Tested microorganisms	Observed results	Reference
Beef carcass	Gaseous ozone atmosphere (0.03 ppm, 6.42 × 10^{-2} mg O_3/m^3)	Mesophilic (M) and psychrotrophic (P) bacteria	Immediately after treatment counts were approximately 2.8 log CFU/g. After 9 days P counts were 2.83 and M counts were 3.1 log CFU/cm^2, while the control was 4.03 (P counts) and 3.90 log CFU/cm^2 (M counts)	Greer and Jones (1989)
Beef	Gaseous ozone (154 mg O_3/m^3)	Total aerobic mesophilic heterotrophic microorganisms and inoculated *Escherichia coli*	Immediately after treatment, there was a 0.7 log cycle decrease in *E. coli* counts and a 2.0 log cycle decrease in total aerobic mesophilic counts	Coll Cardenas et al. (2011)
Ground Hanwoo beef	0.01 mg O_3/h at 4°C for 3 days	7 log CFU/g *Escherichia coli* O157:H7; total aerobic and anaerobic bacteria	Inhibition of *E. coli*, total aerobic and anaerobic bacteria growth during 3 days of storage	Cho et al. (2014b)
Beef	Carbon monoxide and ozone (100% CO; 2% O_3/98% CO; 5% O_3/95% CO; 10% O_3/90% CO) under MAP conditions for 1.5 h	Total viable counts (TVC)	After 45 days storage at 0°C total viable counts, showed lower values. O3 showed an efficient sterilization capacity, and it was proportional to the concentration of O_3	Lyu et al. (2016)
Beef	Ozone pulses ranging between 5 and 10 min, every 30 min for 5 h using concentrations of 280 mg O_3/m^3. And vacuum packaging for storage	Lactic acid bacteria (LAB), mesophilic bacteria, Enterobacteriaceae Inoculated *L. monocytogenes* (10^2 CFU/g tissue)	Immediately after treatment reduction >1 log cycle of LAB, mesophilic and Enterobacteriaceae. Counts of *L. monocytogenes* were below the detection limit after 16 days at 4°C	Gimenez et al. (2021)
Fermented sausages	Ozone concentration was maintained at 0.5 ppm. The ozone treatment was conducted 8 h per day for 4 months	Heterogeneous molds	The applied treatment inhibited the growth of anomalous mold strains, and allowed the growth of the starter culture used, P. nalgiovense	Pirani (2010)
Chicken breasts	Gaseous ozone, >2000 ppm (4.28 × 10^3 mg O_3/m^3) for up to 30 min or 15 min followed by storage under 70% CO_2:30% N_2 (MAP). Storage temperatura = 7°C	*Pseudomona aeruginosa* and *Salmonella infantis*	Reduction of 95% of *P. aeruginosa* and 97% of *S. infantis* counts immediately after ozone treatment. Indigenous coliforms were unaffected. MAP has little further impact. Shelf-life and sensory aspects remained acceptable throughout the storage period of 9 days	Al-Haddad et al. (2005)
Fresh chicken	Gaseous ozone flow of 33 mg/min for 9 min	*L. monocytogenes*	Immediately after treatment a significant decrease (4 log cycles) of *L. monocytogenes* counts	Muthukumar and Muthuchamy (2013)
Chicken breast	Samples were exposed during 1 day to gaseous ozone (10 mg O_3/h in a chamber of 25 × 20 × 20 cm)	Inoculated *Salmonella typhimurium* (G-)	An initial reduction of 0.4 log CFU/g was reported. After 3 days of storage, the control samples reached a value of 8.30 CFU/g, while the ozone-treated samples presented a count of 7.51 CFU/g	Cho et al. (2014b)
Fresh chicken legs	Ozone doses 2, 5, and 10 mg/L, (2 × 10^3, 5 × 10^3 and 10 × 10^4 mg O_3/m^3) and vacuum packaging stored at 4 ± 1 C, for 16 days	Total viable counts *Pseudomonas spp.*, LAB, yeasts and molds, and Enterobacteriaceae	Combination of gaseous ozone (5 and 10 mg/L) and vacuum packaging showed microbial counts <7 CFU/g during 16 days, extending the shelf-life for 6 days compared with the control	Gertzou et al. (2017)
Cooked pork	Combined vacuum cooling with ozone treatment (150 mg O_3/m^3 for 30 min)	Clostridium perfringens (G+)	Increased the dormancy phase, decreased growth rates and prolonged the shelf life by two times. Counts were below 7 log CFU/g for 7 days	Liao et al. (2021)
Turkey Breast Meat	Ozone treatment: 1 × 10^4 mg/m^3, for up to 8 h	Counts of total aerobic mesophilic bacteria, Enterobacteriaceae and yeast-mold	Approximately 2.9, 2.3 and 1.9 log reductions were achieved in total aerobic mesophilic bacteria, Enterobacteriaceae and yeast-mold respectively immediately after treatment	Ayranci et al. (2020)

(Continued on following page)

TABLE 1 (*Continued*) Effect of gaseous ozone concentration on meats.

Type of meat	Gaseous ozone treatment	Tested microorganisms	Observed results	Reference
Fish skin	Gaseous ozone concentrations of 270 mg O_3/m^3	*Pseudomonas putida, Shewanella putrefaciens, Brochothrix thermosphacta, Enterobacter sp.and Lactobacillus plantarum*	Decrease of 1 log CFU/cm^2 of the tested microorganisms was detected after 4 days of storage	Da Silva, Gibbs, and Kirby (1998)
Cod and red shrimp	3 cycles of 5 min (3.5 ppm of gaseous ozone) at days 0, 2. 4 cycles of 10 min (4.7 ppm of ozone) at days 5, 7, 9, and 12	Psychrotrophs, H_2S producing bacteria, *Aeromonas* and *Brochothrix* spp.	*Brochothrix* spp. was detected from the 2nd day Values slowly increased up to almost 4 Log CFU/g *Aeromonas* counts did not differ significantly from the control during storage; after 12 days, counts of H_2S producing bacteria were similar to the control	Aponte et al. (2018)
Scald fish and musky octopus	6 cycles of 5 min (8 ppm of gaseous ozone) at days 0, 2, 5, 7, 9, and 12	Total viable counts (TVC)	Significant differences of TVC were observed, from day 5 of storage at 2°C, in all cases; after 12 days counts were below 5 log CFU/cm^2	Aponte et al. (2018)
Salmon	Ozone doses: 1 mg/m^3 or 3 mg/m^3 Exposure times:5 or 10 min	*Photobacterium*	The more extended treatments showed the largest decrease in microorganism counts (1-1.5 cycles log)	Qian et al. (2022)

CO; 10% O_3/90% CO) under MAP conditions for 1.5 h, and then vacuum packaged. The samples were evaluated after 45 days storage at 0°C and total viable counts, showed lower values after the combined pretreatment.

Gimenez et al. (2021) treated beef samples with ozone pulses ranging between 5 and 10 min duration every 30 min for 5 h, using concentrations of 280 mg O_3/m^3, allowed the reduction of more than 1 log the counts of lactic acid bacteria, mesophilic, Enterobacteriaceae and decreased the counts of inoculated *L. monocytogenes* (10^2 CFU/g tissue) to values below the detection limit for 16 days at refrigerated storage at 4°C.

Pirani (2010) studied the use of gaseous ozone at low concentration to reduce or to stop the development of grey-black spots, caused by heterogeneous molds, on the surface of fermented sausages that are non-acceptable to most consumers. Ozone concentration within the treatment rooms was maintained at 0.5 ppm during the experiments. The ozone treatment was conducted 8 h per day overnight for all the ripening period (4 months). The applied treatment inhibited the growth of anomalous mold strains, and allowed the growth of the starter culture used, *P. nalgiovense*.

Muthukumar and Muthuchamy (2013) used 25 g of the fresh chicken samples that were dipped in 30, 45, and 60 s in deionized water mixed with approximately10^8 CFU/mL of *L. monocytogenes*. Subsequently, the samples were air-dried under a laminar flow hood for 1 h, and were ozonated for 1–9 min at a dose of 33 mg/min. Following each ozonation time, the surviving population of *L. monocytogenes* on the chicken was determined and compared with the non-ozonated samples. The study revealed that ozone at doses of 33 mg/min for 9 min in gaseous phase could be used as an effective method for inactivating 2 × 10^6 CFU/g of *L. monocytogenes* on chicken samples.

Cho et al. (2014b) inoculated chicken breast samples with *S. typhimurium* (G^-) and reported a reduction of 0.4 log CFU/g in samples exposed during 1 day to gaseous ozone (10 mg O_3/h in a chamber of 25 × 20 × 20 cm); counts were lower than in the untreated inoculated meat (7.84 log CFU/g tissue), evidencing the bacteriostatic effect of ozone. After 3 days of storage, the control samples reached a value of 8.30 CFU/g, while the ozone-treated samples presented a count of 7.51 CFU/g.

Gertzou et al. (2017), found that the combination of gaseous ozone (2, 5 and 10 mg/L) and vacuum packaging, extended shelf life of chicken legs under refrigeration for 6 days (5 and 10 mg/L), as compared to single vacuum packaging. *Pseudomonas*, total viable counts (TVC), Enterobacteriaceae and lactic acid bacteria (LAB) counts in fresh meat exceeded 7 log CFU/g tissue after 10 days of storage, while ozone-treated samples (5 and 10 mg/L) were below this value for 16 days.

Jaksch et al. (2004) treated commercial samples of pork meat with ozone in order to determine whether such treatment reduces microbial growth and extends the shelf lifetime of these products. The technique of Proton-Transfer-Reaction Mass Spectrometry (PTR-MS) was used to study volatile emissions with the signal detected at mass 63 (assumed to be a measure for dimethylsulphide) being used as a diagnostic of bacterial activity.

Liao et al. (2021), studied the effect of combining vacuum cooling with an ozone-based re-pressurization process (Invac) on *Clostridium perfringens* (G+). This treatment (150 mg O_3/m^3 for 30 min) increased the dormancy phase, decreased growth rates and prolonged the shelf life of cooked pork by two times. Samples treated with ozone presented counts below 7 log CFU/g tissue for 7 days, while control samples after 4 days exceeded 7 log CFU/g tissue.

Ayranci et al. (2020) studied the effect of gaseous ozone treatment, at a concentration of 10 g O_3/m^3 at different exposure times (2, 4, 6, and 8 h), on total aerobic mesophilic bacteria counts in turkey meat samples. They found that all ozone treatments significantly reduced the initial counts of mesophilic bacteria; the values obtained were between 1.5 and 3 log reductions. With reference to enterobacteria, a decrease of microbial counts by

about 1–1.5 log units was observed in turkey meat when the samples were exposed for 2–4 h and 2.3 units after 6 h.

Fresh fish and marine products are extremely perishable compared to other meats. The hygienic quality of such food rapidly declines due to microbial cross-contamination from various sources, ultimately leading to spoilage (Manousaridis et al., 2005).

Da Silva et al. (1998) analyzed the performance of gaseous ozone in five species of fish bacteria *Pseudomonas* putida, Shewanella putrefaciens, Brochothrix thermosphacta, *Enterobacter* sp. and *Lactobacillus* plantarum, reporting a decrease of 1.0 log CFU/cm_2 when fishes were subjected to an initial ozone treatment (60 min) and a daily exposure (30 min) at concentrations of 270 mg O_3/m^3.

Aponte et al. (2018) studied the effect of ozone treatments (6 cycles of 5 min of 8 ppm ozone at days 0, 2, 5, 7, 9, and 12 of storage) on *Enterobacteriaceae* and *Aeromonas* spp present in different fresh fish products (musk octopus and blanched fish). Ozonation proved to be efficient, with a decrease of around 2 log CFU/g in ozonated musk octopus and less than 4 log CFU/g in ozonized scalded fish.

Qian et al. (2022) studied the effect of gaseous ozone with different doses and exposure times (1 mg/m^3 or 3 mg/m^3 for 5 min and 1 or 3 mg/m^3 for 10 min), on the microbial growth of salmon. The more extended treatments showed the largest decrease in microorganism counts (1–1.5 cycles log).

4 Aqueous ozone treatments

The use of aqueous ozone treatment in the food industry has gained significant importance due to its numerous benefits and versatile applications. Aqueous ozone serves as an effective disinfectant, capable of reducing pathogens, bacteria, viruses, and other microorganisms in meat products, thus greatly enhancing food safety.

Reagan et al. (1996) analyzed trimming and washing of beef carcasses as a method for improving the microbiological quality of meat; they compared treatments using ozonated water or hydrogen peroxide, obtaining a higher reduction in aerobic plate counts for ozone (1.30 and 1.14 log, respectively); however, the use of hot water washing was more effective.

Stivarius et al. (2002) analyzed the effects of beef trimming decontamination with ozone in comparison to chlorine dioxide, on ground beef microbial flora; color and odor characteristics were also studied. Beef trimmings were inoculated with *Escherichia coli* (EC) and *Salmonella Typhimurium* (ST), then treated with either 1% ozonated water for 7 min (7O) or 15 min (15 O), or with 200 ppm chlorine dioxide (CLO) and compared with a control. Trimmings were ground, packaged and sampled at 0, 1, 2, 3, and 7 days of display for EC, ST, coliforms (CO), aerobic plate counts (APC). The 15 min treatment with ozonated water and CLO treatments reduced ($p < 0.05$) all bacterial types evaluated, whereas the 7O treatment reduced ($p < 0.05$) APC and ST.

Novak and Yuan, (2003) studied the effect of aqueous ozone in beef cuts that were inoculated with *Clostridium perfringens* (G^+), *E. coli O157:H7* (G^-) and *L. monocytogenes* (G^+). The samples were washed with ozonated water (3 ppm = 3 mg/L) at 48°C with agitation for 5 min. For each inoculated microorganism, microbial count reductions of 1.28, 0.85, and 1.09 log respectively were reported with ozone treatment.

Castillo et al. (2003) sprayed with an aqueous ozone solution (95 mg/L) beef surfaces inoculated with *E. coli* O157:H7 and *S. typhimurium* (G-) and they have not observed significant differences in microbial counts when results were compared with the application of pure water.

The use of chilled aqueous ozone (temperature = 4.6°C–5.6°C) at a concentration of 12 ppm, applying 90 s of spray every 30 min for 12 h, reduced 1.46 log *E. coli* O157:H7 on the surfaces of fresh beef and 0.99 log aerobic bacteria; however, the treatment did not significantly reduce aerobic bacteria on the surfaces (Kalchayanand et al., 2019).

Jindal et al. (1995) evaluated the efficacy of using ozone during immersion chilling for improving the microbial safety and extending the shelf life of broiler drumsticks. Ozone was dispersed in the chill water, with water continually recirculating in the chill tank. Aqueous ozone was in contact with raw poultry surfaces; initial ozone concentration in chill water ranged between 0.44 and 0.54 ppm during immersion chilling (45 min at 0°C–4°C); the samples were then individually wrapped and stored at 1°C–3°C. Ozone reduced the levels of aerobic plate count, coliforms, and *E. coli* on broiler drumsticks by more than 1.11, 0.91, and 0.90 logs, respectively. Levels of *Pseudomonas aeruginosa*, Gram-negative, and Gram-positive bacteria were reduced by 0.38, 1.11, and 1.14 logs. Ozonation extended the shelf life (product was considered spoiled at ≥ log10 7.0 CFU/cm^2) of broiler drumsticks for as much as 2 days. Microbial reductions noted in poultry chill water were even greater than those on the surface of drumsticks.

Ozonated seawater was used, to inhibit *Vibrio* bacteria from shrimps (Blogoslawski et al., 1993). Chawla et al. (2007) reported that soaking peeled shrimps in ozonated water was more effective than the spray treatment. Soaking shrimp in 3 ppm ozone dissolved in water for 60 s, showed the best results for microbial reduction of total aerobic bacteria and *Pseudomonas* sp.

In a study conducted on salmon fillets involving 1, 2, and 3 spray passes with aqueous ozone solutions at concentrations of 1 mg/L and 1.5 mg/L, it was reported that aerobic bacterial populations were reduced compared to the initial counts under all tested conditions. The most effective reduction (1.05 ± 0.18 log reduction at day 0) occurred when three spray passes were applied using concentrations of 1.5 mg ozone/L. For salmon filets inoculated with *L. innocua*, ozone treatment with three passes of 1 mg/L ozone sprays was effective in significantly reducing ($p \leq 0.05$) *L. innocua* counts (1.17 ± 0.04 log reduction at day 0). They reported that microbial counts were influenced by the number of passes under the spray nozzles, with increasing passes resulting in increasing reductions (Crowe et al., 2012).

De Mendonça Silva and Gonçalves (2017) investigated the efficiency of ozonated water as a disinfectant for removing microorganisms in freshwater fish. Nile tilapia samples (whole and fillets) were immersed in cold water (11°C), without ozone (0 ppm—control) and with ozone (0.5, 1.0, 1.5 ppm) for 0, 5, 10, and 15 min. Microbiological and physicochemical parameters were evaluated. The most efficient ozone concentration to reduce microbiological contamination of the whole tilapia was 1.5 ppm (88.25% of reduction) at 15 min of contact. Ozonated water at 1 and 1.5 ppm showed the greatest reduction (77.2% and 79.49%, respectively) in the fillet treatment.

TABLE 2 Effect of aqueous ozone treatment on different meats.

Type of meat	Aqueous ozone treatment	Tested microorganisms	Observed effects	Reference
Beef carcass	Washing with water and rinsing with ozone (0.3–2.3 ppm)	Aerobic plate counts (APC), *E. coli* (EC)	Immediately after treatment, a reduction of 1.30 and 1.14 log CFU/cm^2 of APC and EC respectively	Reagan et al. (1996)
Beef (brisket)	Ozonated water 0.5%	*E. coli*	Effective in reduction	Gorman et al. (1995)
Beef carcass	Ozonated water 0.5%	Total aerobic plate counts	Times at which samples exceeded 6 log CFU/cm^2 were 11–16 days of treatment	Gorman et al. (1997)
Ground beef	Treatment with 1% ozonated water for 7 min or 15 min	*E. coli* (EC), coliforms (CO), *Salmonella Typhimurium* (ST) and aerobic plate count (APC)	Immediately after 15 min ozone treatment, reductions of 0.44, 0.78 and 0.57 log CFU/g, of CO, ST and APC respectively. Ozone had a residual impact on controlling EC and ST during storage at 4°C for 7 days	Stivarius et al. (2002)
Beef cuts	Samples were washed with ozonated water (3 ppm = 3 mg/L) at 48°C with agitation for 5 min	Inoculation with *Clostridium perfringens* (G+), *E. coli* O157:H7 (G-) and *Listeria monocytogenes* (G+)	Reductions of 1.28, 0.85 and 1.09 log	Novak and Yuan, (2003)
Beef surfaces	Samples were sprayed with aqueous ozone solution (95 mg/L)	Inoculation with *E. coli* O157:H7 and *Salmonella typhimurium* (G-)	Significant differences in microbial counts were not observed	Castillo et al. (2003)
Fabricated beef surfaces	Aqueous ozone (5ppm) followed by heating (55°C)	Enterotoxin producing strains of *Clostridium perfringens*	2.09 log reduction of vegetative cells and 0.95 log reduction of spores immediately after treatment	Novak and Yuan (2004)
Beef surfaces	Aqueous ozone (5 ppm) treatment followed by heating at 45°C–75°C	Enterotoxin producing strains of *Clostridium perfringens*	Immediately after treatment 1.5–2 log CFU/g reduction of *C. perfringens* (vegetative cells)	Novak and Yuan (2004)
Beef surfaces	Spray chilled aqueous ozone (4.6°C–5.6°C and 12 ppm) applied during 90 s every 30 min for 12 h	*E. coli* O157:H7 and aerobic bacteria	Reductions of 1.46 log and 0.99 log	Kalchayanand et al. (2019)
Poultry (drumsticks)	Ozone treatment:0.44 or 0.54 ppm of ozone	Aerobic plate counts (APC), coliforms and *E. coli, Pseudomonas aeruginosa* and total Gram-negative bacteria	Ozone was effective in reductions of 1.11, 0.9 0.91, 0.38 and 1.11 logs respectively	Jindal et al. (1995)
Fresh and frozen chicken meat	Ozonated water (0.5 ppm) for 30 and 45 min	*Staphylococcus aureus*	Immediately after treatment reductions of 2–4 log for 30 and 45 min respectively were observed	Kanaan (2018)
Chicken drumsticks	Six sequential washing and seven sequential spraying cycles with 8 ppm ozonated water	*Salmonella typhimurium and Salmonella choleraesuis* initial load on the surface of the skin was 6.9 (logCFU)/cm2	The complete treatment reduced the bioload of *Salmonella* below the detectable limit	Megahed et al. (2020)
Peeled shrimps	Soaking in 2 ppm and 3 ppm O_3 (ozone dissolved in water) for 60 s	Aerobic bacteria *Pseudomonas sp*	Significantly reduce aerobic spoilage bacteria and *Pseudomonas sp*	Chawla et al. (2007)
Salmon fillets	Aqueous spray treatments of 1 mg/L and 1.5 mg/L ozone (1–3 passes)	*Listeria innocua*	2/3 passes of 1 mg/L aqueous ozone treatment led to 1.17 log reduction at day 0, and after 7 days the difference with control was 0.5 log (final counts 5 log CFU/g)	Crowe et al. (2012)
Nile tilapia samples (whole fish and fillets)	Cold water (11°C), with ozone (0.5, 1.0, 1.5 ppm) for 0, 5, 10 and 15 min	Mesophilic bacteria	In whole tilapia, 1.5 ppm O_3 reduced 88.25% of the microorganisms after 15 min of contact.In fillets, 1 and 1.5 ppm O_3 reduced 77.2% and 79.49%, respectively	De Mendonça Silva and Gonçalves (2017)

Table 2, shows a summary of the effect of aqueous ozone on different microorganisms in various food matrices.

Several studies have analyzed the effect of ozone in combination with other treatments. Delgada et al. (2019) found that the combination of alkaline electrolyzed water and ozonated water (0.68 ± 0.11 mg O_3/L) on goat meat resulted in higher log reductions of *E. coli* (1.03 CFU/mL) compared to ozonated water alone (0.53 CFU/mL).

Megahed et al. (2020) studied the microbial destruction capacity of the aqueous mixture of O_3 and O_3-lactic acid (O_3-LA) under different operating conditions on chicken thighs contaminated with *Salmonella* using sequential soaking and spraying methods. Stefanini et al. (2023) found that the combination of a 5 ppm (5 mg/L) aqueous ozone solution with a 5ppm chlorine solution (Cl + Oz) in tilapia fillets reduced mesophilic bacteria by 0.56 log CFU/g compared to the untreated

control. However, no effect on extending the shelf life compared to the control was observed.

Shrimp samples pretreated by immersion in cold ozonated water (1 ppm, 10 min, 15°C) and chlorinated water (5 ppm, 10 min, 15°C) and then packaged in air (AIR) and in a modified atmosphere (MAP) showed mesophilic counts <1.40 log CFU/g on day 0. On day 3, an increase in total mesophilic counts was found in samples stored in air, while samples stored in MAP remained with values < 1.40 log CFU/g. The highest efficiency in bacterial reduction was observed in the first 3 days of storage in ozone-treated and MAP samples (Gonçalves and Lira Santos, 2018).

5 Effect of ozone treatments on physicochemical and sensory properties

Ozone could affect the physicochemical, sensory and nutritional status of the meat and meat products. The most noticeable effect of ozone was on the surface color of meat samples. According to sensory assessment, ozonation may have a varying impact depending on the meat product: in red meats ozone can oxidize muscle tissues, damage the quality, modifying surface color (undesirable discolorations) and increasing rancidity in fatty tissues.

Ozone effect on physicochemical properties depends on many factors: characteristics of the sample and processing conditions (gaseous or aqueous ozone), concentration, temperature and treatment time. Ozone and other reactive oxygen species (ROS) are strong oxidants that initiate myoglobin oxidation producing metmyoglobin (Bekhit et al., 2013; Khanashyam et al., 2021), and the decrease in CIE a* color parameter, causing discoloration of meat (Mancini and Hunt, 2005).

Gaseous ozone treatment (0.03 ppm) for 9 days at 1.6°C on beef carcass dramatically increased shrinkage and a* value was reduced from 17.8 to 7.38 (Greer et al., 1989).

Stivarius et al. (2002) reported that ground beef samples treated with 1% ozonated water for 7 min or 15 min increased (L*) values while redness (a*) slightly decreased in the shortest treatment of 7 min.

Cho et al. (2014a) studied the effect of gaseous ozone exposure (10×10^{-6} kg O_3/h) at 4°C for 3 days on ground Hanwoo meat in parameters such as thiobarbituric acid reactive substances (TBARS) and color changes. Ozone exposure reduced the CIE a* value of samples over storage time and TBARS values increased from 0.66 at day 1 to 0.79 mg malonaldehyde/kg meat at 3 days storage time.

Gimenez et al. (2021) reported that the treatment with gaseous ozone pulses, lasting between 5 and 10 min each, administered every 30 min for 5 h using concentrations of 280 mg O_3/m^3 on beef, increased L* values compared to the control sample; however, the red color of the meat did not change significantly, with respect to the TBARS values, they reported a final concentration after ozone treatment of 0.7539 ± 0.0370 mg of malonaldehyde/kg meat.

In the studies of Cho et al. (2014a), Ayranci et al. (2020) and Giménez et al. (2021), TBARS values of ozone-treated samples did not exceed 1 mg of malonaldehyde per kg meat, that is the acceptable sensory threshold limit for exhibiting rancid flavor.

Significant changes in L*, a* and b* values of ozone-treated chicken breast samples (gaseous ozone at concentration of 10 mg O_3/h) were reported by Cho et al. (2014b), showing a decrease of L* and a* and an increase of b* during storage.

Muhlisin et al. (2016) reported significant increases in TBARS for duck and to a lesser extent chicken filet, stored for 4 days at 4°C, under a flow of gaseous ozone (10 mg O_3/m^3/h) in which the ozone generator had an automatic timer that was set to on for 15 min and off for 105 min.

Megahed et al. (2020) reported that the treatment on chicken drumstick with aqueous ozone (10 serial washes of 4 min, each one with water containing 8 ppm ozone) did not cause any significant change in color.

Ayranci et al. (2020) report that in turkey breast meat the treatment with gaseous ozone (10 g/m^3) for up to 8 h, at 22°C caused significant changes in the different parameters when the initial values were compared with those obtained after 8 h treatment; thus, TBARS increased from 0.06 to 0.37 mg of malonaldehyde/kg meat and color parameters changed. L* increased from 34.43 to 41.97 and a* decreased from 2.08 to 0.35.

Crowe et al. (2012) working on salmon fish reported that a spray of aqueous ozone (concentration 1.0 and 1.5 mg/L and 1, 2, and 3 passes under spray) did not disrupt the characteristic pigmentation of salmon; no significant differences in a* values on salmon treated samples and controls were observed, indicating that ozone did not induce bleaching of the red pigments.

In the study by De Mendonça Silva and Gonçalves (2017), conducted on Nile tilapia, ozonated water treatment did not influence the pH or color of the fillets. However, a slight triggering of the lipid oxidation process was observed, as evidenced by an increase in the TBARS value.

In the fresh fish and bivalve mollusk, ozone application suppresses the smell characteristic which sometimes can be disagreeable, giving a healthful aspect to seafood. It is advisable to consider that ozone, in this case, does not have to be used to mask the low quality of the products (Gonçalves, 2009).

6 Final remarks

The review highlights ozone effectiveness in controlling microorganism growth, improving the quality of meat, and extending shelf life. Ozone, as a non-thermal disinfectant, is eco-friendly and replaces conventional chemical sanitizers. Its efficacy varies depending on application method, food matrix, and microbial strain requiring tailored treatment parameters.

The disinfection properties of ozone is attributed to its oxidation-reduction potential (2.08 eV); the intracellular reactive oxygen species are responsible for the detrimental effects in nucleic acids and bacterial cell lysis, which under stress produces leakage of intracellular content. Ozone has effects on DNA damage because of the oxidation of double bonds by singlet oxygen; the oxidation of membrane glycoproteins and/or glycolipids is also produced. Two possible primary mechanisms of microorganism inactivation by ozone treatment were proposed: the oxidation of sulfhydryl groups and amino acids of peptides, proteins, and enzymes to produce smaller peptides, and the oxidation of polyunsaturated fatty acids to acid peroxides.

Using ozone as a decontaminating agent, instead of traditional agents such as chlorine, is justified by its significant oxidative properties. It is about 50% stronger than chlorine, and shows a broad spectrum of antibacterial activities. Even though ozone does not leave any residues due to a quick decomposition of its structure, some restrictions should be applied in the case of human exposure to this gas.

Ozone treatments showed a decrease in bacterial counts of specific pathogens, such as *S. typhimurium, L. monocytogenes,* and *E. coli,* in different types of meat. Results reported in Tables 1, 2 indicate in some cases, that growth was not observed during meat storage and ozone caused a decrease in bacterial growth parameters extending the shelf life.

The application of ozone treatment simultaneously with other technologies would allow reducing ozone concentration and treatment times, thus maximizing the desired effect on nutritional properties and microbial safety of foods. Chickens and seafood showed longer shelf life when ozone was combined with other preservation methods, emphasizing the potential synergistic or additive effects of ozone with refrigeration or vacuum packaging. Sensitivity of microorganisms to ozone treatment varies according to the method of applying ozone, the food matrix (content of organic compounds), the microbial species and strain. However, a marked difference in the sensitivity of various microorganisms to ozone was evidenced, a situation that needs to correctly be established, defining the ozone dose, the duration of contact, the treatment conditions, the form of ozone application, etc., depending on the type of product being treated for its effective and safe use in food processing. Since each ozone application is different, pilot testing should be conducted before commercial application is initiated. While gaseous ozone is more effective in reducing microbial populations, high doses or prolonged exposure can alter physicochemical properties of meats, affecting color and lipid oxidation. Despite potential sensory changes, ozone-treated products generally meet quality standards.

Aqueous ozone is usually used more for the decontamination of surfaces such as poultry carcasses, while gaseous ozone is used for cut/processed meats. Regarding the processes, gaseous ozone presents a greater reduction of microbial population.

Treating food with ozone offers a number of significant advantages, making it a highly beneficial, environmentally friendly and economically viable option as an antimicrobial agent.

In the food sector, ozone has proven to be a viable technique, because it does not require extremely high temperatures; on the contrary, it is more effective at low temperatures because ozone is more soluble under this condition, making it an energy-saving technique. In addition, the absence of chemical residues reduces waste disposal costs and the need for final rinsing. This technology not only enhances food safety and product quality, but also provides economic benefits by extending the shelf life of food and reducing losses due to decomposition and spoilage.

Ozone has several additional benefits; its excellent antimicrobial capacity, supported by its superior oxidation potential, prevents bacteria from developing resistance. Moreover, because ozone safely reverts to oxygen and leaves no chemical residues, it ensures a safe and environmentally friendly process. Its on-site production using electrical energy eliminates the need to store hazardous chemicals, contributing to a safer working environment. It also reduces waste water disposal costs by leaving no residue to require special treatment.

In addition, ozone improves indoor air quality by destroying airborne microorganisms, preventing cross-contamination of pathogens. Its recognition as safe for food processing and its ability to eliminate pathogens are extra factors supporting its use in the food industry. With its proven performance and safety, ozone has become an effective and economical disinfection solution, offering benefits in terms of product quality and environmental sustainability.

Author contributions

BG: Writing–original draft, Writing–review and editing, Investigation. NZ: Conceptualization, Funding acquisition, Investigation, Resources, Supervision, Writing–review and editing, Project administration. NG: Investigation, Writing–original draft, Writing–review and editing.

Funding

The author(s) declare that financial support was received for the research, authorship, and/or publication of this article. The financial support from Universidad Nacional de La Plata (UNLP), Project 11/I 246 is gratefully acknowledged.

Acknowledgments

The authors gratefully acknowledge the financial support from Universidad Nacional de La Plata (UNLP), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Comisión de Investigaciones Científicas (CIC) de la Provincia de Buenos Aires, Agencia Nacional de Promoción Científica y Tecnológica (ANPCYT)—ARGENTINA.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The author(s) declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

Publisher's note

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