*Journal of Food Protection, Vol. 82, No. 6, 2019, Pages 940–947*
doi:10.4315/0362-028X.JFP-18-489


**Mini-Review**

# Ozone-Based Interventions To Improve the Microbiological Safety and Quality of Poultry Carcasses and Parts: A Review

**CARMEN CANO,[1] YULIE MENESES,[1,2] AND BYRON D. CHAVES[1]***

[1]*Department of Food Science and Technology and* [2]*Daugherty Water for Food Global Institute, University of Nebraska-Lincoln, Lincoln, Nebraska 68588, USA*

MS 18-489: Received 9 October 2018/Accepted 24 January 2019/Published Online 15 May 2019

## ABSTRACT

Poultry meat represents an important part of the U.S. economy and diet. However, it remains one of the food categories responsible for the most outbreak-associated foodborne illness cases. Therefore, the food safety and public health communities continue to examine appropriate antimicrobial interventions to reduce product contamination and the risk of foodborne disease. Ozone treatment has become an attractive microbial decontamination option for food products including poultry because of its antimicrobial properties and minimal effects on quality. The objective of this review is to summarize the current scientific literature on the application of ozone in chicken carcasses and parts. Fourteen primary research studies met the inclusion criteria. Ozone treatment achieved microbial population reductions of 0.2 to 0.94 log CFU/mL of rinsate, 0.08 to 1.28 log CFU/cm$^2$, and 0.0 to 5.3 log CFU/g for specific target microbial populations. Among the factors that influenced treatment efficacy were ozone concentration, exposure time, and the microbial population of interest. Studies indicate that ozone treatment can be optimized to extend the shelf life of poultry products without a significant effect on physicochemical and sensory qualities, which makes it a potential suitable hurdle to improve food safety. Further research is required to better understand the effect of ozone on poultry-borne pathogens like *Salmonella* spp. and *Campylobacter* spp. and to validate its application and scale-up in industrial settings. This review identifies important knowledge gaps that may guide future studies about this novel decontamination technology.

## HIGHLIGHTS

- Ozone treatment achieved microbial population reductions.
- Gaseous ozone was most commonly used on poultry parts.
- Carcasses were treated exclusively with aqueous ozone or ozonated water.
- Ozone treatment can extend poultry product shelf life without significant quality effects.

Key words: Chicken carcasses; Chicken parts; Decontamination; Ozone; Poultry

Foodborne illness remains an important public health burden. According to the U.S. Centers for Disease Control and Prevention, from 2009 to 2015, investigators identified a specific food vehicle as a causal agent in 2,442 outbreaks of foodborne illness (42%), and the food vehicle belonged to a single food category in 1,281 outbreaks (22%) of 5,760 total reported outbreaks during that period *(7)*. Chicken (123 outbreaks, or 10%) was one of the three food categories most commonly implicated in foodborne illness outbreaks in the United States, after fish (222 outbreaks, 17%) and dairy (136 outbreaks, 11%). However, chicken was the food category responsible for the most outbreak-associated foodborne disease cases (3,114 illnesses, 12%) during the same period, followed closely by pork (2,670 illnesses, 10%) and seeded vegetables (2,572 illnesses, 10%) *(7)*. In the United States, the pairing of *Campylobacter* and poultry has been estimated to rank as the pathogen–food combination with the highest annual disease burden, with a yearly cost of illness of $1.257 million, corresponding to 608,231 illnesses, 6,091 hospitalizations, and 55 deaths per annum *(2)*. Similarly, the pairing of *Salmonella enterica* and poultry ranks fourth among the most common food–pathogen combinations, with an annual cost of illness of $693 million, corresponding to more than 215,000 illness cases, more than 4,000 hospitalizations, and nearly 80 deaths per year *(2)*. The morbidity and mortality caused by *Salmonella* and *Campylobacter* continue to be a challenge *(40, 41)*. The introduction, adoption, and implementation of mandatory hazard analysis and critical control points for the meat and poultry industry, as well as prevalence performance standards set by the U.S. Department of Agriculture Food Safety and Inspection Service (USDA-FSIS), have helped reduce the public health burden of *Salmonella* and *Campylobacter* over the past 20 years *(20)*. Despite great strides in foodborne pathogen control, the food safety and public health communities continue to examine how food becomes contaminated with pathogens and how to develop

* Author for correspondence. Tel: 402-472-2196; Fax: 402-472-1693; E-mail: byron.chaves-elizondo@unl.edu.

appropriate interventions and regulations to minimize the risk of foodborne illness for the consumer *(20)*.

The United States is the world's largest producer and second-largest exporter of poultry meat, and the 2019 production is expected to increase year over year, largely owing to expanded slaughter *(32)*. Chicken consumption in the United States has increased in the last 6 years from 55 lb of boneless, trimmed, and edible portion per capita in 2011 to 63.7 lb per capita in 2017 *(31)*. In 2015, an estimated 40% of chicken on a ready-to-cook weight basis were sold as cut-up parts, while 11% were sold whole and 49% were sold as further processed products, such as chicken nuggets or rotisserie birds *(38)*. The shift from commercializing primarily whole carcasses to mostly cut-up parts has brought new food safety challenges to the poultry industry. For example, for the 2017 to 2018 period, the estimated national prevalence of pathogens in chicken parts (legs, breasts, and wings) in relation to production volume was 14.83% for *Salmonella* spp. and 1.88% of *Campylobacter* spp., compared with only 4.90% prevalence of *Salmonella* spp. and 0.58% of *Campylobacter* spp. on whole carcasses after chilling *(36)*. A similar prevalence report in 2012 led to revised performance standards for reducing *Salmonella* and *Campylobacter* in poultry products, which were published by the USDA-FSIS in January 2015 *(33)*. Presumably, the processing of chicken carcasses exposes dermal skin tissues, providing a new surface niche for bacterial colonization during subsequent steps *(27)*. This may partially explain the differences in qualitative and quantitative microbial profiles of whole carcasses versus individual parts.

Epidemiological data indicate that there is a need to reduce the incidence of *Salmonella* and *Campylobacter* among consumers and in the general population. Consequently, it is critical that the industry acts to reduce the prevalence of poultry-borne foodborne pathogens with effective antimicrobial intervention technologies. The USDA-FSIS has listed interventions that poultry establishments may incorporate into their production processes to lower the prevalence of these pathogens in their products, including preharvest and processing interventions *(33, 35)*. However, it concluded that adding antimicrobial solutions to poultry parts would be the most likely response from establishments that do not meet the proposed performance standards, especially because most facilities were not applying antimicrobials to raw poultry parts at the time of the survey *(33)*. The objectives of this mini-review are to (i) summarize the existing scientific literature on the application of ozone-based interventions for decontamination of poultry carcasses and parts; (ii) provide a critical analysis of the factors that influence its efficacy in poultry products; and (iii) identify knowledge gaps and potential opportunities for future research.

## OZONE AS AN ANTIMICROBIAL INTERVENTION

Under USDA-FSIS Directive 7120.1 Rev. 46 *(35),* ozone is considered a processing aid with antimicrobial applications in all meat and poultry products when used in accordance with current industry standards of good manufacturing practices. Although ozone has been used for food decontamination purposes in the past, the applications were limited by its toxicity, volatility, reactivity, and occupational concerns *(16)*. However, advances in ozone generation, dissolution, and mitigation into the environment have resulted in safer applications for the reduction of pathogenic and spoilage organisms in food commodities *(16)*. Ozone was deemed generally recognized as safe in the United States in 1997, which encouraged its use over a spectrum of foods as an alternative to chlorine and other chemical treatments. Because it is highly unstable, it decomposes rapidly into oxygen without leaving residues on or in the product *(4, 19)*. Ozone is a stronger oxidizer than chlorine and causes changes in cell permeability that lead to cellular lysis and leakage. Its decomposition in solution produces free radicals such as hydroperoxyl, hydroxyl, and superoxide, which also have great oxidizing power *(11)*. As a result, it oxidizes the double bonds of fatty acids in cell walls and plasma membranes, especially in the lipoprotein and lipopolysaccharide layers of gram-negative bacteria such as *Salmonella, Campylobacter,* and *Escherichia coli (4, 16, 17)*. Presumably, this is the principal mechanism of action of ozone, and the reduction in microbial populations translates into higher microbiological safety and quality. The antimicrobial mechanism of ozone has been reviewed elsewhere *(4, 17)*.

Ozone has attracted attention for its minimal effects on nutritional, chemical, and physical properties of food compared with other chemical treatments *(9)*. This is important for producers because consumers look for wholesome, high-quality, and safe foods that are minimally processed *(9)*. Because ozone cannot be generated and stored, its widespread industrial use was limited before the commercial development of portable in situ ozone generators, which are now available *(39)*. Consequently, the application of ozone-based interventions to improve food safety and quality must be objectively assessed.

## PREPARATION OF THE MINI-REVIEW

The European Food Safety Authority's "Application of Systematic Review Methodology to Food and Feed Safety Assessments to Support Decision Making" *(8)* was used as the guidance document to carry out this review. The closed-framed, descriptive question "What are the factors that affect the effectiveness of ozone treatments for the microbiological decontamination of poultry carcasses and parts?" was formulated to guide the review. Then, the inclusion criteria for primary research studies were defined as follows: any scientific article reporting ozone-based treatment of poultry carcasses or parts (including breasts, drumsticks, thighs, and wings) and its qualitative and/or quantitative effect on microbial population, either native or artificially inoculated, regardless of the publication date or geographical origin.

Scoping of the scientific literature was performed after formulation of the review question. Inquiries were conducted on Scopus and ScIELO using the descriptors "ozone AND poultry," "ozone AND broiler," and "ozone AND chicken" to search article titles, abstracts, and key words. After initial exploration of the primary literature in April 2018, the search was repeated the first week of every month

until November 2018. All relevant primary research articles identified in English or Spanish were retrieved in full text through the University of Nebraska–Lincoln library system. Only scientific publications reporting qualitative or quantitative microbiological reductions directly attributed to ozone-based treatments were used for data extraction. In total, 106 articles were found with the descriptors "ozone AND poultry," 75 were found with "ozone AND chicken," and 29 were found with "ozone AND broiler." All abstracts were read, and only those articles that met the inclusion criteria were used in the following phase. After elimination of duplicates, 14 full-text scientific publications *(1, 5, 9, 10, 12, 13, 15, 22–24, 29, 30, 39, 42)* were selected and read in their entirety before data extraction. The following information was identified from each publication: poultry matrix, target microbial population (*Campylobacter, Salmonella,* indicators, and others), ozone-based treatment (aqueous, gaseous, and other) and treatment conditions (concentration, exposure time, pressure, temperature, etc.), initial population counts, final population counts, and population reduction directly attributed to ozone-based treatments. If reported as raw data, population counts were converted to logarithmic scale to facilitate potential comparisons among studies.

The publication date of the 14 included studies ranged from 1979 to 2017, but most articles (78.5%, 11 of 14) were published from 2002 onward. The primary research studies on chicken parts reported trials with breasts (skin on or skinless) and drumsticks (with skin); no studies were found concerning other chicken parts. Nine of the articles worked with the native microflora of chicken, while the other five worked with artificially inoculated samples. Gaseous ozone and aqueous ozone were the most common treatments, used in seven and six studies, respectively, while ozone in dry ice was used in a single study.

## ANTIMICROBIAL EFFECTIVENESS OF OZONE-BASED INTERVENTIONS

Several authors have evaluated the effectiveness of ozone treatments for decontamination of poultry carcasses and parts. Yang and Chen *(42)* reported reductions of 1.0, 1.0, and 0.8 log CFU/cm$^2$ on mesophilic, psychrotrophic, and psychrophilic bacterial counts, respectively, for thighs and breasts treated with ozone gas at 3.88 ppm for 20 min. Similarly, Gertzou et al. *(12)* found that 10 ppm of gaseous ozone applied for 1 h could reduce total viable counts in chicken legs almost 1.5 log CFU/g, which extended the shelf life for 4 days. In addition, Jindal et al. *(15)* reported 1.11-log CFU/cm$^2$ reductions in aerobic plate counts when chicken drumsticks were immersed in ozonated water at 0.44 to 0.54 ppm for 45 min. Vadhanasin et al. *(39)* found that *Salmonella* prevalence was lower when carcasses were treated with ozone at 125 ppm for 40 to 50 min (3 of 20 samples, 15%) than when treated with chlorine for the same time (15 of 66 samples, 22.7%), although the concentration of chlorine was not stated, and neither were the quantitative reductions in *Salmonella* populations. However, Trindade et al. *(30)* found that counts of psychrotrophs (3.2 log CFU/mL of rinsate) and coliforms (4.7 log CFU/mL of rinsate) for samples treated with aqueous ozone at 1.5 ppm for 45 min were not significantly different from the corresponding counts (3.1 and 4.9 log CFU/mL of rinsate) for samples treated with aqueous chlorine at 1.5 ppm for 45 min. Other studies have reported quantitative reductions of under 1 log. For example, Fabrizio et al. *(9)* concluded that immersion chilling in ozonated water at 10 ppm was not effective enough to significantly reduce *Salmonella* Typhimurium, *E. coli,* and total coliforms on broiler carcasses, because reductions of only 0.74, 0.78, and 0.81 log CFU/mL of rinsate were estimated. The reductions were not deemed of practical significance by the researchers *(9)*. Sheldon and Brown *(29)* reported reductions of 0.66, 1.06, and 0.72 log most probable number per 10 mL of rinsate for aerobic plate counts, coliforms, and *Salmonella,* respectively, but only 0.2 log CFU/mL of rinsate for psychrotrophic plate counts after immersing chicken carcasses in water with 3.0 to 4.5 ppm of ozone for 45 min. Overall, the authors considered the ozone treatment suitable to reduce spoilage and pathogenic bacteria on poultry carcasses *(29)*. Among the challenge studies reported, Al-Haddad et al. *(1)* found that gaseous ozone (>2,000 ppm) was effective at reducing *Salmonella* in artificially inoculated chicken breasts up to 1.28 log CFU/cm$^2$ when applied for 30 min. A presumably larger effect was observed by Muthukumar and Muthuchamy *(24),* who achieved *Listeria monocytogenes* reductions of up to 6.3 log CFU/g when inoculated chicken pieces were treated with gaseous ozone at 33 mg/min for 9 min. However, the differences in methodologies and in inherent bacterial responses make it difficult to directly compare the sizes of the effects attributed to ozone treatment.

Some of the most novel applications include the use of ozone in combination with other interventions, such as freeze-drying or dry ice, or as an integral part of storage. Fratamico et al. *(10)* reported that ozone introduced into dry ice pellets achieved a 1.3-log CFU/g reduction of artificially inoculated *Campylobacter jejuni* on chicken breast after 24 h of storage, and the authors considered this reduction of practical significance for the industry *(10)*. According to Muhlisin et al. *(22),* gaseous ozone exposure during 3 days of refrigeration storage was also effective in reducing *Salmonella* Typhimurium and total aerobic and anaerobic bacterial counts on chicken breasts by 0.79, 1.07, and 1.01 log CFU/g, respectively. Based on the research of Cantalejo et al. *(5),* the ozone treatment of freeze-dried chicken breasts (0.6 ppm for 10 min) resulted in a 1-log CFU/g reduction of aerobic plate counts at the end of 8 months of storage compared with the samples treated with lyophilization alone, deeming the combination treatment effective at extending the product shelf life.

## PRODUCT FACTORS INFLUENCING INTERVENTION EFFECTIVENESS

Most studies included reported information for only one type of poultry product, making it difficult to compare the effect of the poultry matrix on treatment effectiveness. In the only research article that reported on more than one product, Muhlisin et al. *(23)* stored both chicken breast and duck breast pieces under ozone flow for 4 days. Ozone significantly ($P < 0.05$) reduced aerobic counts in chicken on day 1 of storage from 3.40 to 2.14 log CFU/g, but a

significant reduction of aerobic counts on duck breast was not observed until day 3 (0.22-log CFU/g reduction on day 1 and 1.59-log CFU/g reduction on day 3). Furthermore, ozone had a negative effect on quality parameters of duck breast, such as lipid oxidation, antioxidant enzyme activity, and surface color. This difference was explained by the higher amount of fat in duck breast (6 g per 100-g edible portion), compared with chicken breast (1 g per 100-g edible portion), which results in undesirable oxidation reactions that lower the amount of ozone available to attack microorganisms *(21, 23)*.

Because of the wide differences in treatment conditions in the different articles surveyed, it is difficult to ascertain the effect of the presence of skin or bones on the effectiveness of ozone interventions. The reported population reductions on skin-on products (carcasses, breasts, thighs, and drumsticks) ranged from 0.2 to 1.06 log CFU/mL of rinsate, while the reductions on skinless products (breasts and thighs) ranged from 0 to 2.65 log CFU/g. Bone-in products, which include carcasses, thighs, breasts, and drumsticks, experienced reductions of 0.2 to 2.1 log CFU/g, while boneless chicken breasts had reductions from 0 to 2.65 log CFU/g. More information is needed to characterize the effect of skin and bones on ozone-based treatment effectiveness.

## PROCESS FACTORS INFLUENCING INTERVENTION EFFECTIVENESS

Higher concentrations of ozone and longer exposure times correlate with higher antimicrobial activity across a range of food categories, such as vegetables, meat, dairy, juices, and spices *(4)*. In the selected studies, exposure times varied for aqueous and gaseous treatments. Aqueous ozone was usually applied as an immersion or spray washing step, and exposure times ranged from 1 to 45 min *(9, 15, 29, 30, 39, 42)*. However, gaseous ozone was used to treat chicken carcasses for 1 min to 3 days by storing carcasses in chambers with ozone generators *(1, 5, 12, 13, 22–24)*.

In general, the combination of exposure time and ozone concentration determines the ozone dose. Cantalejo et al. *(5)* treated skinless chicken breasts with 0.4, 0.6, and 0.72 ppm of gaseous ozone before freeze-drying and storage. They reported significantly lower ($P < 0.05$) aerobic counts for samples treated with 0.60 and 0.72 ppm of ozone for 30 min compared with 0.4 ppm for 30 min. Gertzou et al. *(12)* found a similar trend in chicken legs treated with 2, 5, or 10 ppm of gaseous ozone for 1 h. The only concentration that significantly extended the shelf life of the product was 10 ppm from 5 or 6 days to 12 days. Likewise, Khadre et al. *(16)* reported higher inactivation of vegetative cells of *Alicyclobacillus acidocaldarius* when aqueous suspensions were treated with higher doses of ozone in a continuous reactor. Based on generalized results, we can hypothesize that a similar trend may be observed in poultry matrixes for spoilage and pathogenic bacteria. However, limiting factors for effectiveness may include fat content and presence of organic matter in the working dispersion, and the effect of these and other factors have yet to be comprehensively evaluated.

Three articles reported studying the effect of exposure time on ozone effectiveness. Al-Haddad et al. *(1)* treated artificially inoculated, skin-on chicken breasts with gaseous ozone at more than 2,000 ppm for 0, 1, 3, 5, 10, 20, and 30 min. The reductions in *Salmonella* Infantis and *Pseudomonas aeruginosa* were time dependent, but even the 30-min treatment did not eradicate *Salmonella* cells. Muthukumar and Muthuchamy *(24)* performed a similar experiment, exposing raw chicken meat inoculated with *L. monocytogenes* at three levels (4.92, 5.04, and 6.30 log CFU/g) to ozone gas at 33 mg/min. The reduction increased with the exposure time, with the highest level of *L. monocytogenes* reduced to undetectable levels at the longest time tested (9 min). It is expected that longer exposure times allow for more extensive contact between ozone and the microorganisms present in the sample, leading to higher population reductions. The trend was also observed in native microflora of chicken breasts by Cantalejo et al. *(5),* who reported lower total aerobic bacteria counts for samples treated with 0.4 ppm of ozone for 120 min compared with samples treated for 30 or 60 min. However, there was no significant difference between samples treated for 10 min and those treated for 30 min, suggesting that a minimum ozone exposure time threshold must be met before the effect of exposure time can be measured. That threshold has yet to be established. A treatment with a longer exposure time and a higher concentration of ozone would seem better suited for decontamination; however, practical considerations such as ozone toxicity, cost, feasibility, and quality effects must be taken into account when designing a decontamination process *(4)*.

## EFFECT OF MICROBIAL FACTORS

Several studies have reported microbial reductions after ozone-based treatment of poultry products. Overall, most bacterial groups studied were susceptible. For example, Jindal et al. *(15)* reported up to 1-log CFU/cm$^2$ reductions in native populations of aerobic plate counts, coliforms, and *E. coli* for chicken drumsticks immersed in ozonated water at 0.44 to 0.54 ppm at 0 to 4°C for 45 min. Similar logarithmic reductions for artificially inoculated *Salmonella* Typhimurium and *P. aeruginosa* on skin-on chicken breasts were reported by Al-Haddad et al. *(1)*. However, they did not observe a significant reduction in the native coliform population. This may be attributed to the different attachment levels between artificially inoculated and native microbial populations. Higher reductions of microbial populations in drumsticks and chicken breasts by different antimicrobial treatments have been observed when the microbial attachment time is shorter *(14),* supporting the preceding hypothesis.

Three of the included studies used artificial inoculation with *Salmonella* spp., one used *C. jejuni,* and one used *L. monocytogenes*. Al-Haddad et al. *(1)* reported a 1.28-CFU/cm$^2$ *Salmonella* reduction from 1.98 to 0.70 log CFU/cm$^2$ in skin-on chicken breasts exposed to more than 2,000 ppm of gaseous ozone for 30 min. Fabrizio et al. *(9)* observed a 0.74-log CFU/mL of rinsate reduction (2.71 to 1.97 log CFU/mL of rinsate) in *Salmonella* counts in chicken carcasses after being treated with 10 ppm of aqueous ozone

for 45 min. In addition, Muhlisin et al. *(22)* reported a 0.79-log CFU/g reduction (from 8.30 to 7.51 log CFU/g) in *Salmonella* counts in chicken breast pieces stored in refrigerated chambers with ozone compared with the control pieces stored only under refrigeration. In the first two cases, the initial inoculation levels were low, but even at high initial concentrations, ozone-based treatments reduced less than 1 log of the population. Muthukumar and Muthuchamy *(24)* used three initial inoculum levels of *L. monocytogenes* (4.91, 5.04, and 6.30 log CFU/g) achieved by varying the dipping time of the chicken samples into the bacterial dispersion. A 1-log CFU/g reduction was achieved after 3 min of gaseous ozone exposure at 33 mg/min for the two lower inoculum levels. After 3 min of gaseous exposure, the samples with the highest initial inoculum showed a 2.15-log CFU/g reduction in *L. monocytogenes* counts. Finally, Fratamico et al. *(10)* observed a 1.3-log CFU/g reduction in *C. jejuni* counts on skinless chicken breasts after 24 h of storage in dry ice with 20 ppm of ozone. Additional studies are needed to draw conclusions about the effectiveness of ozone interventions on samples inoculated with pathogens at low or high concentrations.

Of the 14 studies included in the review, 9 used freshly harvested chickens as a product matrix, 5 used refrigerated carcasses or parts purchased commercially, and 1 used previously frozen chicken parts. In addition, 2 studies used irradiation treatment to reduce the background microbial flora before artificially inoculating the chicken samples. All these treatments exert an effect on the physiological state of the bacterial populations. For example, it has been reported that cold-stressed *Salmonella* and *Enterococcus faecium* cells were more sensitive than unstressed cells to postchilling antimicrobial dipping treatments on chicken carcasses *(18)*. Among the studies included in the review, Yang and Chen *(42)* observed similar initial counts of aerobic bacteria (3.5 log CFU/cm$^2$) in frozen chicken parts to those found by Jindal et al. *(15)* (2.9 log CFU/cm$^2$) in freshly harvested chicken drumsticks. Both studies report similar log reductions (1.0 and 1.2 log CFU/cm$^2$); however, Yang and Chen *(42)* used a different ozone dose (3.88 ppm of aqueous ozone for 20 min) than that used by Jindal et al. *(15)* (0.44 to 0.54 ppm of aqueous ozone for 45 min). The differences in ozone concentrations, exposure times, and sample pretreatment, as well as sampling methods, make it difficult to draw conclusions about the effect of cold stress on the susceptibility of bacterial pathogens to ozone.

Differences were also reported between gram-negative and gram-positive populations. In two separate studies, Gertzou et al. *(12, 13)* observed significant reductions for gram-negative populations such as *Pseudomonas* spp. and *Enterobacteriaceae,* but not for lactic acid bacteria (LAB). Cantalejo et al. *(5)* also reported lower reductions in counts of LAB than in aerobic plate counts for ozone-treated samples. Little information is available on the sensitivity of LAB to ozone. Lower initial reductions in populations of LAB than in *Pseudomonas* populations in shucked mussels and rainbow trout have been reported *(19, 25),* while reductions in LAB and *Pseudomonas* achieved in beef were not significantly different *(3)*. The fate of this bacterial population is particularly important in vacuum-packaged poultry products, where LAB are the main population responsible for spoilage. Greater susceptibility of gram-negative bacteria to ozonated water in vitro compared with gram-positive bacteria has been reported *(6, 28)*. The difference may be attributed to the presence of lipoprotein and lipopolysaccharide layers in gram-negative bacteria's cell envelopes *(17)*. Polyunsaturated fatty acids, membrane-bound enzymes, glycoproteins, and glycolipids in gram-negative bacteria are all susceptible to oxidation, which leads to leakage of cell contents and eventual lysis *(16)*. However, with the current body of scientific evidence, it is not possible to make broader generalizations regarding the susceptibility of one bacterial group over the other.

## PRODUCT SHELF LIFE AND QUALITY

Besides food safety applications, ozone treatments may affect shelf life and quality of poultry products. The typical mesophilic aerobic plate counts for fresh broiler chicken carcasses range from 3 to 4 log CFU/g, with values of 7 log CFU/g considered spoiled products *(12)*. In the selected studies, ozone treatment reduced the initial aerobic plate count on poultry products, slowed outgrowth during storage, or both, resulting in 1 to 4 additional days of acceptable microbiological quality *(5, 9, 12, 13, 15, 23, 42)*.

As a strong oxidizer, ozone could have potential negative effects on the fat portion of poultry products, which is rich in polyunsaturated fatty acids *(30)*. Oxidative changes in food products can be analyzed with a 2-thiobarbituric acid (TBA) measurement. Sheldon and Brown *(29)* found significantly lower TBA numbers for breast, thigh, and skin tissues treated with 3.0 to 4.5 ppm of aqueous ozone for 45 min than for the control tissues treated with tap water. Only ozone-treated drumstick tissue had higher TBA numbers than its control counterpart *(29)*. Trindade et al. *(30)* reported that the thiobarbituric acid reactive substances (TBARS) index for chicken carcasses treated with ozone at 1.5 ppm of aqueous ozone for 45 min was similar to that of chlorine-treated carcasses (1.5 ppm of chlorine solution for 45 min). A slight increase in the TBARS index was observed during the storage time; however, the values were too low to be perceived by sensory analysis *(30)*. It can be concluded that the ozone doses used in these studies (1.5 to 4.5 ppm of aqueous ozone for up to 45 min) were appropriate to avoid lipid oxidation.

The importance of ozone exposure level on lipid oxidation was demonstrated by Muhlisin et al. *(22),* who stored chicken breasts for 3 days in a chamber with an ozone generator that ran for 15 min and then turned off for 45 min before running again. For the first 2 days, there was no significant difference in TBARS values for ozone samples compared with the control. However, ozone significantly increased lipid oxidation at 3 days of storage. This could be the result of the weakening of antioxidant enzyme activity by ozone exposure or a direct attack of ozone on the lipids in the cells, causing irreversible damage to fatty acids in the cell membrane *(22)*. In further experiments, the ozone generator was programmed to run for 15 min and then turn off for 105 min, lowering the total ozone exposure. With this setup, a significant difference in

lipid oxidation as TBARS values was not observed until day 4 of storage *(23)*.

The sensory effects of physical, chemical, and microbial changes in poultry because of ozone treatment should also be taken into account. The pH of chicken legs during 12 days of storage was not affected by doses of 2, 5, or 10 ppm of gaseous ozone *(12),* and Trindade et al. *(30)* found no difference between the pH of chicken carcasses treated with aqueous ozone and those treated with chlorine, both at 1.5 ppm for 45 min at 4°C. Furthermore, Trindade et al. *(30)* found no significant difference in lightness (L*) and yellowness (b*) parameters for carcass skin color between ozone and chlorine treatments or storage times. The redness (a*) values showed a slight decrease over storage time, but this was not perceived by consumers in the sensory evaluation. In addition, Sheldon and Brown *(29)* reported similar results, with no significant differences in L*a*b* (CIE) color reflectance values for skin on the breast, drumstick, and back among hot, water-chilled, or ozone-chilled carcasses. A washing effect was noticed visually in water-chilled and ozone-chilled carcasses, described as a slight reduction in the carcass redness compared with the hot carcasses. For ozone-treated chicken legs, Gertzou et al. *(12)* found that L*a*b* (CIE) parameters decreased over the 12 days of refrigerated storage. However, the decrease was not significantly different between untreated and ozone-treated samples. In another study, trained panelists found that ozone-treated, freeze-dried, and rehydrated chicken meat retained an acceptable appearance and odor during 8 months of storage, while the non–ozone-treated control was not sensory acceptable after 4 months *(5)*. Hardness, juiciness, and chewiness of freeze-dried, rehydrated, and cooked samples, both ozone-treated and untreated, decreased gradually. Samples treated with higher doses of ozone or treated for a longer time were deemed unacceptable earlier in the storage period than those treated with lower doses or for a shorter time *(5)*. This underlines the importance of optimizing ozone treatment for all relevant product characteristics, not only food safety.

A different effect was observed in refrigerated, aerobically packaged fresh chicken drumsticks, where a higher ozone dose (10 ppm for 1 h) resulted in a longer sensory shelf life than lower ozone doses (2 and 5 ppm for 1 h), ensuring acceptable odor, texture, appearance, and taste for at least 2 additional days in each category. The total shelf life for this treatment was 10 days, a 4-day extension from that of the untreated control *(12)*. After combining this treatment with vacuum packaging, sensory shelf life was further extended to 16 days *(13)*. In this experiment, three inhibition or inactivation methods—low temperature, ozone, and vacuum packaging—were used at suboptimal levels to maintain microbial safety of the food product without compromising the sensory quality.

## OZONE-BASED TREATMENTS WITHIN AN INTEGRATED HURDLE TECHNOLOGY

Because of its reported minimal effects on sensory properties, ozone may be incorporated as a hurdle in an integrated food preservation approach. Hurdle technology is defined as using two or more inhibition or inactivation methods at suboptimal levels in which they are synergistically more effective *(5)*. In the research articles included in this review, ozone treatment was combined with freeze-drying, modified atmosphere packaging, vacuum packaging, and even dry ice for poultry applications, resulting in a higher reduction in microbial populations in samples with combined treatments compared with samples with only one treatment *(5, 9, 10, 13)*. Cantalejo et al. *(5)* investigated the effects of ozone treatment on the shelf life of freeze-dried chicken meat fillets stored at room temperature. Three ozone doses (0.4, 0.6, and 0.72 ppm) and four exposure times (10, 30, 60, and 120 min) were used. Although the initial loads of aerobic plate and LAB were not significantly different between the ozonated and the nonozonated samples, a significant reduction ($P < 0.05$) in counts because of ozone treatment was observed when sampling after 4, 6, and 8 months of storage. After 8 months of storage, mesophilic counts for ozone-treated samples were 6.8 and 3.26 log CFU/g lower than for the frozen meat and freeze-dried meat controls, respectively. Fratamico et al. *(10)* used ALIGAL Blue Ice, which incorporates ozone in dry ice pellets, to store chicken breast samples inoculated with *C. jejuni*. After 24 h of storage, the ALIGAL Blue Ice treatment exhibited a reduction of 1.3 log CFU/g, while the dry ice and wet ice controls exhibited reductions of 0.8 and 0.5 log CFU/g, respectively, pointing toward a potential synergistic treatment effect.

Gertzou et al. *(12, 13)* developed two similar studies in which different ozone concentrations (2, 5, and 10 ppm) were applied to fresh chicken legs for 1 h. In the first study, the chicken legs were packaged in air-permeable polyamide and polyethylene bags and refrigerated, while in the second study, vacuum packaging was used. Shelf life was determined based on microbiological, physicochemical, and sensory evaluations over a 12-day period for aerobically packaged samples and over a 16-day period for vacuum-packaged samples. The controls, aerobically packaged and vacuum-packaged chicken leg meat without ozone treatment, were found to be acceptable for 6 and 10 days, respectively. Ozone treatment (10 ppm) extended the shelf life of aerobically packaged meat to 10 days and that of vacuum-packaged meat to 16 days. Therefore, ozone could be considered to have increased shelf life by 4 days, while vacuum packaging increased shelf life by 6 days compared with the aerobically packaged control. The combined treatment increased shelf life by 10 days, hinting at a positive synergistic effect between ozone treatment and vacuum packaging.

## KNOWLEDGE GAPS, PERSPECTIVES, AND CONCLUSIONS

No studies have reported the use of ozone for decontamination of chicken wings. Chicken wings are the third-highest-grossing cut in the fresh meat chicken category in the United States, representing $881 million in 2017, only behind breasts and thighs. Wings also occupy the third place in the deli-prepared chicken category at $590 million in 2017 *(37)*. Lower decontamination effects on chicken wings compared with whole broiler carcasses have been reported for antimicrobials such as peroxyacetic acid

and lactic acid *(18)*. Presence of skin may also play a role, because greater reductions in *Salmonella* counts have been observed in skin-off chicken thighs compared with skin-on chicken thighs when treated with organic acids *(27)*. This highlights the need for testing ozone effectiveness under multiple product and process scenarios. Similarly, no information is available on the use of ozone for decontamination of ground poultry products.

Of the 14 research articles evaluated, only 3 carried out a challenge study with *Salmonella* inoculation, of which 2 used *Salmonella* Typhimurium and 1 used *Salmonella* Infantis. Further studies are required to better evaluate the effect of ozone treatment on different *Salmonella* serotypes. The USDA-FSIS *(34)* reported the following *Salmonella* serotypes as the most commonly identified in young chicken carcasses (broilers) in the United States in 2014: *Salmonella* Kentucky (60.8% of positive samples), *Salmonella* Enteritidis (13.6%), *Salmonella* Typhimurium (7.7%), *Salmonella* Infantis (6.5%), and *Salmonella* Heidelberg (3.4%). The remaining 8.0% of positive samples were associated with other serotypes. Although *Salmonella* Kentucky from chicken carcasses is not among the serotypes commonly associated with human illness in the United States, *Salmonella* Enteritidis is the most common serotype associated with human illness. Paul et al. *(26)* reported a significant difference in susceptibility to chlorine among 12 poultry-associated *Salmonella* serotypes when varying concentrations of chicken organic matter are present. We can hypothesize that a similar difference in susceptibility to ozone is possible; therefore, interventions must be designed that target the most common serotypes under different processing and operational conditions.

In addition, only one of the studies evaluated the effect of ozone on chicken parts artificially inoculated with *Campylobacter* spp., and only one used *Listeria* spp. Although the prevalence in whole carcasses and in chicken parts is not as high as that of *Salmonella, Campylobacter* spp. remain a substantial health burden in the United States and around the world *(2)*. It is important to investigate ozone effectiveness against this pathogen in chicken parts challenge studies, because the intestinal tract of birds is identified as the main reservoir of this microorganism. In addition, nonpathogenic surrogate organisms should be evaluated. Surrogates mimic the behavior of pathogens and can be used in actual poultry processing facilities to verify that antimicrobials are effective, without raising biosafety concerns. *E. faecium* has been used successfully as a *Salmonella* surrogate for antimicrobial interventions on broilers in small-scale poultry processing settings *(18)*. It would be worthwhile to evaluate its suitability as a surrogate for ozone interventions. Furthermore, assays that promote cell recovery after ozone treatment should be investigated to avoid overestimating the effectiveness of the treatment.

In conclusion, 14 studies applying ozone treatments to reduce microbial populations in chicken carcasses or chicken parts were identified that fulfilled the review inclusion criteria. Ozone treatment achieved microbial population reductions of 0.20 to 0.94 log CFU/mL of rinsate, 0.08 to 1.28 log CFU/cm$^2$, and 0.0 to 5.3 log CFU/g, depending on the concentration, exposure time, and type of microbial population. Four challenge studies were identified; the remaining 10 studied the effect on native microflora of chicken meat. Breasts and drumsticks were the parts most frequently studied, but no studies on chicken wings were reported. Gaseous ozone was most commonly used on parts, while carcasses were treated exclusively with aqueous ozone or ozonated water. Studies indicate that ozone-based treatments can be optimized to extend the shelf life of poultry products without a significant effect on physicochemical and sensory qualities. Further studies are required to better understand the effect of ozone on poultry-borne foodborne pathogens like *Salmonella* spp. and *Campylobacter* spp. and to validate its application and scale-up in industrial settings.

## REFERENCES


1. Al-Haddad, K. S. H., R. A. S. Al-Qassemi, and R. K. Robinson. 2005. The use of gaseous ozone and gas packaging to control populations of *Salmonella* Infantis and *Pseudomonas aeruginosa* on the skin of chicken portions. *Food Control* 16:405–410.
2. Batz, M. B., S. Hoffmann, and J. G. Morris, Jr. 2012. Ranking the disease burden of 14 pathogens in food sources in the United States using attribution data from outbreak investigations and expert elicitation. *J. Food Prot*. 75:1278–1291.
3. Botta, C., I. Ferrocino, M. C. Cavallero, S. Riva, M. Giordano, and L. Cocolin. 2018. Potentially active spoilage bacteria community during the storage of vacuum packaged beefsteaks treated with aqueous ozone and electrolyzed water. *Int. J. Food Microbiol*. 266:337–345.
4. Brodowska, A. J., A. Nowak, and K. Śmigielski. 2017. Ozone in the food industry: principles of ozone treatment, mechanisms of action, and applications: an overview. *Crit. Rev. Food Sci. Nutr*. 58:2176–2201.
5. Cantalejo, M. J., F. Zouaghi, and I. Pérez-Arnedo. 2016. Combined effects of ozone and freeze-drying on the shelf-life of broiler chicken meat. *LWT - Food Sci. Technol*. 68:400–407.
6. Devatkal, S. K., P. Jaiswal, A. Kaur, and V. Juneja. 2016. Inactivation of *Bacillus cereus* and *Salmonella enterica* serovar Typhimurium by aqueous ozone: modeling and UV-VIS spectroscopic analysis. *Ozone Sci. Eng*. 38:124–132.
7. Dewey-Mattia, D., K. Manikonda, A. J. Hall, M. E. Wise, and S. J. Crowe. 2018. Surveillance for foodborne disease outbreaks—United States, 2009–2015. *Morb. Mortal. Wkly. Rep*. 67:1–11.
8. European Food Safety Authority. 2010. Application of systematic review methodology to food and feed safety assessments to support decision making. *EFSA J*. 8:1637.
9. Fabrizio, K. A., R. R. Sharma, A. Demirci, and C. N. Cutter. 2002. Comparison of electrolyzed oxidizing water with various antimicrobial interventions to reduce *Salmonella* species on poultry. *Poult. Sci*. 81:1598–1605.
10. Fratamico, P. M., V. Juneja, B. A. Annous, V. Rasanayagam, M. Sundar, D. Braithwaite, and S. Fisher. 2012. Application of ozonated dry ice (ALIGAL Blue Ice) for packaging and transport in the food industry. *J. Food Sci*. 77:M285–M291.
11. Fröhling, A., and O. Schlüter. 2015. Flow cytometric evaluation of physico-chemical impact on gram-positive and gram-negative bacteria. *Front. Microbiol*. 6:939.
12. Gertzou, I., P. Drosos, I. Karabagias, and K. Riganakos. 2016. Combined effect of ozonation and packaging on shelf life extension of fresh chicken legs during storage under refrigeration. *J. Food Sci. Technol*. 53:4270–4277.
13. Gertzou, I. N., I. K. Karabagias, P. E. Drosos, and K. A. Riganakos. 2017. Effect of combination of ozonation and vacuum packaging on shelf life extension of fresh chicken legs during storage under refrigeration. *J. Food Eng*. 213:18–26.
14. İlhak, O., G. İncili, and H. Durmuşoğlu. 2018. Effect of some chemical decontaminants on the survival of *Listeria monocytogenes*

and *Salmonella* Typhimurium with different attachment times on chicken drumstick and breast meat. *J. Food Sci. Technol*. 55:3093–3097.
15. Jindal, V., A. L. Waldroup, R. H. Forsythe, and M. J. Miller. 1995. Ozone and improvement of quality and shelf-life of poultry products. *J. Appl. Poult. Res*. 4:239–248.
16. Khadre, M. A., A. E. Yousef, and J. Kim. 2001. Microbiological aspects of ozone applications in food: a review. *J. Food Sci*. 66:1242–1252.
17. Kim, J., A. E. Yousef, and S. Dave. 1999. Application of ozone for enhancing the microbiological safety and quality of foods: a review. *J. Food Prot*. 62:1071–1087.
18. Lemonakis, L., K. Li, J. M. Adler, and C. Shen. 2017. Microbiological quality assessment and validation of antimicrobials against unstressed or cold-stress adapted *Salmonella* and surrogate *Enterococcus faecium* on broiler carcasses and wings. *Poult. Sci*. 96:4038–4045.
19. Manousaridis, G., A. Nerantzaki, E. K. Paleologos, A. Tsiotsias, I. N. Savvaidis, and M. G. Kontominas. 2005. Effect of ozone on microbial, chemical and sensory attributes of shucked mussels. *Food Microbiol*. 22:1–9.
20. McEntire, J., D. Acheson, A. Siemens, S. Eilert, and M. Robach. 2014. The public health value of reducing *Salmonella* levels in raw meat and poultry. *Food Prot. Trends* 34:386–392.
21. Mozdziak, P. 2004. Species of meat animals/poultry, p. 1296–1302. *In* W. K. Jensen (ed.), Encyclopedia of meat sciences. Academic Press, Oxford.
22. Muhlisin, M., Y. Cho, J. H. Choi, T. Hahn, and S. K. Lee. 2015. Bacterial counts and oxidative properties of chicken breast inoculated with *Salmonella* Typhimurium exposed to gaseous ozone. *J. Food Saf*. 35:137–144.
23. Muhlisin, M., D. T. Utama, J. H. Lee, J. H. Choi, and S. K. Lee. 2016. Effects of gaseous ozone exposure on bacterial counts and oxidative properties in chicken and duck breast meat. *Korean J. Food Sci. Anim. Resour.* 36:405–411.
24. Muthukumar, A., and M. Muthuchamy. 2012. Optimization of ozone in gaseous phase to inactivate *Listeria monocytogenes* on raw chicken samples. *Food Res. Int*. 54:1128–1130.
25. Nerantzaki, A., A. Tsiotsias, E. Paleologos, I. Savvaidis, E. Bezirtzoglou, and M. Kontominas. 2005. Effects of ozonation on microbiological, chemical and sensory attributes of vacuum-packaged rainbow trout stored at 4±0.5°C. *Eur. Food Res. Technol*. 221:675–683.
26. Paul, N. C., T. S. Sullivan, and D. H. Shah. 2017. Differences in antimicrobial activity of chlorine against twelve most prevalent poultry-associated *Salmonella* serotypes. *Food Microbiol*. 64:202–209.
27. Ramirez-Hernandez, A., M. M. Brashears, and M. X. Sanchez-Plata. 2018. Efficacy of lactic acid, lactic acid–acetic acid blends, and peracetic acid to reduce *Salmonella* on chicken parts under simulated commercial processing conditions. *J. Food Prot*. 81:17–24.
28. Restaino, L., E. W. Frampton, and J. B. Hemphill. 1995. Efficacy of ozonated water against various food-related microorganisms. *Appl. Environ. Microbiol*. 61:3471–3475.
29. Sheldon, B. W., and A. L. Brown. 1986. Efficacy of ozone as a disinfectant for poultry carcasses and chill water. *J. Food Sci*. 51:305–309.
30. Trindade, M. A., M. M. Kushida, N. D. Montes Villanueva, D. U. dos Santos Pereira, and C. E. Lins de Oliveira. 2012. Comparison of ozone and chlorine in low concentrations as sanitizing agents of chicken carcasses in the water immersion chiller. *J. Food Prot*. 75:1139–1143.
31. U.S. Department of Agriculture, Economic Research Service. 26 July 2018. Livestock and meat domestic data. Available at: https://www.ers.usda.gov/data-products/livestock-meat-domestic-data/livestock-meat-domestic-data/. Accessed 31 July 2018.
32. U.S. Department of Agriculture, Economic Research Service. 2018. Livestock, dairy, and poultry outlook: July 2018. U.S. Department of Agriculture.
33. U.S. Department of Agriculture, Food Safety and Inspection Service. 2015. Changes to the *Salmonella* and *Campylobacter* verification testing program: proposed performance standards for *Salmonella* and *Campylobacter* in not-ready-to-eat comminuted chicken and turkey products and raw chicken parts and related agency verification procedures and other changes to agency sampling. *Fed. Regist*. 80:3940–3950.
34. U.S. Department of Agriculture, Food Safety and Inspection Service. 2016. Serotypes profile of *Salmonella* isolates from meat and poultry products. January 1998 through December 2014.
35. U.S. Department of Agriculture, Food Safety and Inspection Service. 2018. Safe and suitable ingredients used in the production of meat, poultry and egg products. FSIS Directive 7120.1 Rev. 46.
36. U.S. Department of Agriculture, Food Safety and Inspection Service. 30 June 2018. Sampling results for FSIS regulated products. Available at: https://www.fsis.usda.gov/wps/portal/fsis/topics/data-collection-and-reports/microbiology/sampling-project-results. Accessed 2 August 2018.
37. U.S. National Chicken Council. 24 January 2018. Americans to eat 1.35 billion chicken wings for Super Bowl. Available at: https://www.nationalchickencouncil.org/americans-eat-1-35-billion-chicken-wings-super-bowl/. Accessed 8 August 2018.
38. U.S. National Chicken Council. 2018. How broilers are marketed. Available at: https://www.nationalchickencouncil.org/about-the-industry/statistics/how-broilers-are-marketed/. Accessed 31 July 2018.
39. Vadhanasin, S., A. Bangtrakulnonth, and T. Chidkrau. 2004. Critical control points for monitoring salmonellae reduction in Thai commercial frozen broiler processing. *J. Food Prot*. 67:1480–1483.
40. Williams, M. S., E. D. Ebel, N. J. Golden, and W. D. Schlosser. 2014. Temporal patterns in the occurrence of *Salmonella* in raw meat and poultry products and their relationship to human illnesses in the United States. *Food Control* 35:267–273.
41. Williams, M. S., N. J. Golden, E. D. Ebel, E. T. Crarey, and H. P. Tate. 2015. Temporal patterns of *Campylobacter* contamination on chicken and their relationship to campylobacteriosis cases in the United States. *Int. J. Food Microbiol*. 208:114–121.
42. Yang, P. P. W., and T. C. Chen. 1979. Effects of ozone treatment on microflora of poultry meat. *J. Food Process. Preserv.* 3:177–185.