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Review

Application of Ozone for Enhancing the Microbiological Safety and Quality of Foods: A Review

JIN-GAB KIM, AHMED E. YOUSEF,* AND SANDHYA DAVE

Department of Food Science and Technology, The Ohio State University, 2121 Fyffe Road, Vivian Hall, Columbus, Ohio 43210, USA

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ABSTRACT

Ozone (O_3) is a strong antimicrobial agent with numerous potential applications in the food industry. High reactivity, penetrability, and spontaneous decomposition to a nontoxic product (i.e., O_2) make ozone a viable disinfectant for ensuring the microbiological safety of food products. Ozone has been used for decades in many countries and recently, the generally recognized as safe (GRAS) status of this gas has been reaffirmed in the United States. Ozone, in the gaseous or aqueous phases, is effective against the majority of microorganisms tested by numerous research groups. Relatively low concentrations of ozone and short contact time are sufficient to inactivate bacteria, molds, yeasts, parasites, and viruses. However, rates of inactivation are greater in ozone demand-free systems than when the medium contains oxidizable organic substances. Susceptibility of microorganisms to ozone also varies with the physiological state of the culture, pH of the medium, temperature, humidity, and presence of additives (e.g., acids, surfactants, and sugars). Ozone applications in the food industry are mostly related to decontamination of product surface and water treatment. Ozone has been used with mixed success to inactivate contaminant microflora on meat, poultry, eggs, fish, fruits, vegetables, and dry foods. The gas also is useful in detoxification and elimination of mycotoxins and pesticide residues from some agricultural products. Excessive use of ozone, however, may cause oxidation of some ingredients on food surface. This usually results in discoloration and deterioration of food flavor. Additional research is needed to elucidate the kinetics and mechanisms of microbial inactivation by ozone and to optimize its use in food applications.

Sanitizers such as hypochlorite solutions and quaternary ammonium compounds have been used in food-processing facilities to control contaminant microorganisms, particularly those causing foodborne diseases. Use of some sanitizers has been limited or banned (e.g., formaldehyde) because of the potential health hazards. On the other hand, the need for potent antimicrobial agents has increased in recent years due to increasing disease outbreaks and emergence of new foodborne pathogens. Illnesses arising from the presence of *Escherichia coli* O157:H7 in frozen ground beef patties and burgers *(30)*, *Listeria monocytogenes* in wieners *(31)*, and hepatitis A in frozen strawberries *(29)* have renewed interest in effective control measures. Therefore, the food industry is in search of disinfectants that are effective against common and emerging pathogens and safe to use in many specific applications of food processing. One such compound is ozone (O_3) that has been utilized as a sanitizer in many European water treatment plants since the beginning of this century *(73)*.

There are many advantages of using ozone as a potent oxidizing agent in food and other industries. It is potentially useful in decreasing the microbial load, the level of toxic organic compounds, the chemical oxygen demand, and the biological oxygen demand in the environment. Ozone converts many nonbiodegradable organic materials into biodegradable forms. The molecule decomposes spontaneously to oxygen; thus, using ozone minimizes the accumulation of inorganic waste in the environment *(92)*. The high oxidizing power and spontaneous decomposition also make ozone a viable disinfectant for ensuring the microbiological safety and quality of food products.

Up to the beginning of this century, ozone had been tested for the preservation of food and food ingredients such as milk, meat products, gelatin, casein, and albumin *(47)*. Hill and Rice *(88)* noted that ozone was applied for the purification and artificial aging of alcoholic beverages including wine and spirits, disinfection of brewing and cider manufacturing facilities, odor control, and medical therapy. However, most known applications dealt with treatment of drinking water *(25)* and municipal and industrial waste water *(180)*.

In the past, application of ozone in the food industry in the United States was limited. It had been used primarily for the removal of iron, manganese, color, tastes, and odors in water *(147)*. In 1982, the U.S. Food and Drug Administration affirmed that ozone is generally recognized as safe (GRAS), with specific limitations, for use as a disinfectant in bottled water *(58)*. The U.S. Department of Agriculture permitted recycling of reconditioned water in poultry chillers *(185)*. Recently, an expert panel in the United States affirmed ozone as a GRAS substance *(75)* for broad food

* Author for correspondence. Tel: 614-292-7814; Fax: 614-292-0218; E-mail: yousef.1@osu.edu.

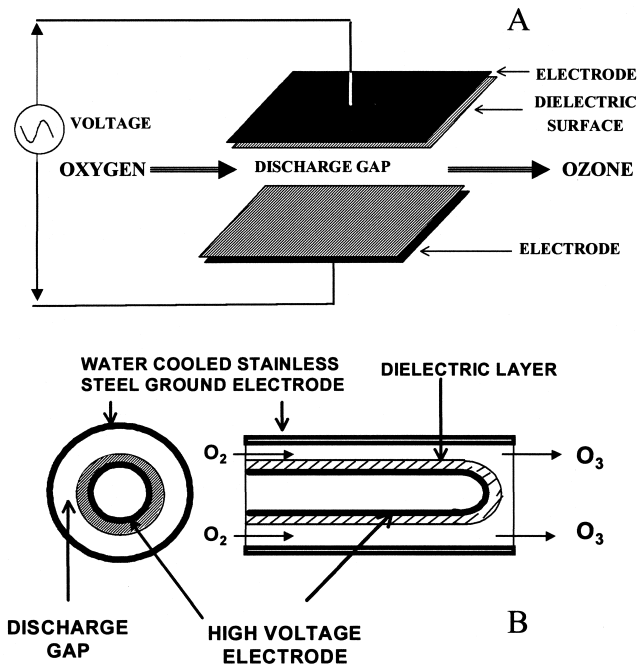

FIGURE 1. *Conceptual design of corona discharge ozone generator (adapted from Rosen* (160)*). (A) Basic configuration, (B) tube-type generator unit.*

applications. Because the U.S. Food and Drug Administration had no objection to this affirmation, ozone now can be used as a disinfectant or a sanitizer in food processing in the United States. These regulatory developments triggered interest in ozone applications among academic researchers and food processors. Therefore, a current and comprehensive review of literature will be valuable in assessing ozone applicability in the modern food industry.

GENERATION, REACTIVITY, AND DECOMPOSITION OF OZONE

An allotropic modification of oxygen, ozone is a bluish gas with pungent and characteristic odor. It has a molecular weight of 48, boiling point of −111.9°C, and melting point of −192.7°C at 1 atm *(133).* Ozone weighs ca. 0.135 lb/ft^3. The oxidation potential of ozone is high (−2.07 V) compared to that of hypochlorous acid (−1.49 V) or chlorine (−1.36 V) *(19).*

Ozone is formed naturally in the stratosphere in small amounts (0.05 mg/liter) by the action of solar UV irradiation on oxygen. A small amount of ozone is also formed in the troposphere as a by-product of photochemical reactions between hydrocarbons, oxygen, and nitrogen that are released from automobile exhausts, industries, forests, and volcanic action. However, the gas produced is very unstable and decomposes quickly in the air *(92).*

When used in industry, ozone is usually generated at the point of application and in closed systems. Ozone is produced at low concentrations (0.03 ppm) from oxygen in the air by radiation of 185-nm wavelength, emitted by high transmission UV lamps *(54).* The corona discharge method has been used most widely to produce large amounts of ozone (Fig. 1). When a high-voltage alternating current is applied across a discharge gap in the presence of air or oxygen, it excites oxygen electrons and thus induces splitting of oxygen molecules. Atoms from split oxygen combine with other oxygen molecules to form ozone, O_3. Ozone production varies depending on voltage, current frequency, dielectric material property and thickness, discharge gap, and absolute pressure within the discharge gap. To optimize ozone production, an efficient heat-removal system is essential. Dried air is passed through a high-voltage current along the discharge gap, thus, converting oxygen into ozone at concentrations up to 4% by weight. The use of pure oxygen is recommended over dried air to maximize the yield of ozone. Dried gas is used to minimize the corrosion of metal surfaces due to nitric acid deposits produced from wet gas inside the generator *(160).*

In addition to photochemical and electric discharge methods, ozone can be produced by chemical, thermal, chemonuclear, and electrolytic methods *(92).* A new approach in producing ozone has been implemented by Lynntech, Inc. (College Station, Tex.) *(128).* This is an electrochemical procedure in which water is split into hydrogen and oxygen atoms by electrolysis. Hydrogen molecules are separated from the gas and water mixture and the oxygen atoms combine to form ozone and diatomic oxygen. The manufacturer claims their system produces ozone at concentrations that are three to four times higher (10 to 18% ozone in the gas-mixture output) than those attainable by corona discharge.

In the upper atmosphere, high-energy UV irradiation helps degrade ozone molecules. Ozone is converted to oxygen in the process and absorbs the UV energy before it reaches the earth's surface *(19).* Levy *(123)* postulated that the photolysis of ozone to oxygen atoms could lead to the generation of the hydroxyl radical (·OH), a key reactive species during the decomposition process. In addition to UV irradiation, high pH, hydrogen peroxide (H_2O_2), and activated carbon enhance the degradation of ozone *(99).*

Ozone decomposes in solution in a stepwise fashion, producing in turn hydroperoxyl (·HO_2), hydroxyl (·OH), and superoxide (·O_2^-) radicals *(1, 80, 89).* 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. According to Hoigne and Bader *(89),* the rate constants for reactions of OH radical with many substrates are very high. Hence, these radicals are consumed preferentially by dissolved species before they encounter dispersed particles such as microorganisms. This occurs even when concentrations of molecular solutes are smaller than those of the particles. In many systems, however, OH radicals react with solutes to form secondary intermediates of lower reactivity (for example, peroxy radicals) that may survive until they encounter a dispersed particle. Decomposition of ozone is so rapid in the water phase of foods that its antimicrobial action may take place mainly at the surface *(89).*

MEASUREMENT OF OZONE

The analytical methods for the determination of ozone can be grouped into physical, physicochemical, and chem-

ical methods. Physical methods are based on measuring particular ozone properties, such as the intensity of absorption in the UV, visible, or infrared region of the spectrum. The physicochemical methods measure physical effects of ozone reaction with different reagents; such effects include chemiluminescence or heat of the reaction. Chemical methods measure the quantity of the reaction products that are released when ozone reacts with an appropriate reagent (e.g., KI or HI) or the reduction in the molecular weight of a polymer. These methods differ in sensitivity and accuracy *(1).*

One of the chemical methods is the indigo colorimetric method *(5)* that was approved by the committee on standard methods for the examination of water and wastewater in 1988 *(2).* In this method, ozone adds across the carbon–carbon double bond of sulfonated indigo dye and decolorizes it. The change in absorbance is determined spectrophotometrically. This method is subject to fewer interferences than most of the colorimetric methods and all iodometric procedures *(71).* For accurate determination of gaseous ozone, the UV spectrophotometric method should be used.

ANTIMICROBIAL ACTION OF OZONE

Inactivation mechanisms. Reactions of ozone with various chemical compounds in aqueous systems occur in two different and coexisting modes, one involving direct reactions of molecular ozone and the other being a free radical-mediated destruction mode *(175).* Singlet oxygen is a likely intermediate reactive species in the biochemical damage caused by ozone *(103).* These multiple mechanisms may apply also to the destructive effect of ozone on bacteria. However, Hunt and Marinas *(93)* found recently that *E. coli* was inactivated primarily by molecular ozone.

Giese and Christenser *(70)* suggested that the bacterial cell surface is the primary target of ozone activity. Scott and Lesher *(170)* detected the leakage of cell contents with ozone treatment. They proposed the double bonds of unsaturated lipids in the cell envelope as the primary site of attack. Murray et al. *(137)* assumed that lipoprotein and lipopolysaccharide layers of gram-negative bacteria would be subjected first to attack by ozone that results in a change in cell permeability, eventually leading to lysis.

According to Komanapalli and Lau *(117),* viability of *E. coli* K-12 was unaffected by short-term exposure (1–5 min) to 600 ppm ozone gas but membrane permeability was compromised. With longer exposures, up to 30 min, cell viability decreased, with a progressive degradation of intracellular proteins. According to Bancroft and Richter *(8),* ozone causes cellular proteins to flocculate. Bringman *(20)* suggested that chlorine selectively destroyed certain enzymes, whereas ozone acted as a general protoplasmic oxidant. Sykes *(181)* concurred with Bringman *(20)* about the cause of cell destruction by ozone. Ingram and Haines *(96)* found a general destruction of the dehydrogenating enzyme systems in *E. coli* after treatment with ozone and proposed that death of the cell may result from interference with the respiratory system. Barron *(10)* suggested that the oxidation of sulfhydryl groups (SH- to S-S) in the enzyme is the principal cause of death. Ozone caused a more rapid decrease in β-galactosidase activity in the cytoplasm than alkaline phosphatase activity in the periplasm of *E. coli (182).*

Ozone may inactivate microorganisms by causing damage to their genetic material. In studies by Prat et al. *(152)* and Scott *(169)* on DNA of *E. coli,* the pyrimidine bases were modified by ozonation, with thymine being more sensitive to ozone than cytosine and uracil. Different mechanisms were proposed to explain the inactivation of viruses by ozone. Kim et al. *(109)* examined tritiated f_2 bacteriophage and its RNA after exposure to ozone. RNA was released from the phage particles during ozonation, and the treated phage had reduced infectivity for spheroplasts. Electron microscopic examination showed that the phage coat was broken by ozonation into many protein subunits and that the specific adsorption of the phage to host pili was inversely related to the extent of phage coat breakage. Roy et al. *(162),* however, observed that the damage to the viral nucleic acid is the major cause of the inactivation of poliovirus 1 (Mahoney). Ozone not only damaged the viral RNA but also altered polypeptide chains of the viral protein coat.

Inhibitory spectrum: bacteria. Ozone inactivates numerous bacteria that include gram-negative and gram-positive and both vegetative cells and spore forms (Table 1). It is not feasible to compare the sensitivity of bacteria to ozone using results from different sources; effectiveness of ozone varies appreciably with minor changes in experimental variables. Selected studies, however, are presented to illustrate the effectiveness of ozone against various bacterial species.

Finch et al. *(61)* determined the extent of inactivation of *E. coli* using ozone doses of 4.4 to 800 μg/liter at contact times of 30 to 120 s. They reported 0.5- to 6.5-log decreases in counts of *E. coli,* depending on the ozone dose and contact time. *Pseudomonas putrefaciens* was added to a pilot-scale water recycling system where ozone was maintained at 1.5 ppm *(136).* The population of *P. putrefaciens* decreased 3 log after 5 min and 6 log after 20 min of exposure. Bactericidal action of ozone depends on the medium into which bacteria are present. Dave et al. *(44)* showed that a *Salmonella* Enteritidis population, in distilled water, decreased 6 log at a low concentration of ozone (1.5 ppm). However, when broiler skin was inoculated with *Salmonella* Enteritidis and exposed to an ozone–air mixture (8%, wt/wt) for 15 s, approximately 1 log reduction in population of the pathogen was observed *(153).* Antimicrobial effects of ozonated water in a recirculating concurrent reactor, against different bacterial species, were evaluated *(157).* Death rates among the gram-negative bacteria (*Salmonella* Typhimurium, *E. coli, P. aeruginosa,* and *Yersinia enterocolitica*) were not significantly different, whereas among gram-positive bacteria, *L. monocytogenes* was significantly more sensitive than either *Staphylococcus aureus* or *Enterococcus faecalis.* Kim *(111)* determined the effectiveness of ozone against foodborne microorganisms such as *P. fluorescens, Leuconostoc mesenteroides, L. monocytogenes,* and *E. coli* O157:H7 in a batch-type reaction system. He

TABLE 1. *Inactivation of bacteria by ozone*

Bacterium	Inactivation (log_{10})	Treatment time (min)	Concentration (mg/liter)	pH	Temp. (°C)	Medium	Reactor type	Reference
Bacillus cereus	>2.0	5	0.12		28	O_3 demand-free water		*22*
B. cereus (spores)	>2.0	5	2.29		28	O_3 demand-free water		*22*
Escherichia coli	4.0	1.67	0.23–0.26	7	24	O_3 demand-free water	Continuous flow	*56*
E. coli	3.0	19	Initial 2.2, residual 0.06	7.5	16	Raw wastewater	Continuous flow	*100*
E. coli	2.0	0.1	0.53	6.8	1	Phosphate buffer	Batch	*60*
Legionella pneumophila	>4.5	20	0.32	7	24	Distilled water	Batch	*50*
Mycobacterium fortuitum	1.0	1.67	0.23–0.26	7	24	O_3 demand-free water	Continuous flow	*56*
Pseudomonas fluorescens	>2.0	0.25						*27*
Salmonella Enteritidis	1.0	0.25	8% (wt/wt)		25	Broiler carcass	Ozone gas	*153*
Salmonella Typhimurium	4.3	1.67	0.23–0.26	7	24	O_3 demand-free water	Continuous flow	*56*
Staphylococcus aureus	>2.0	0.25		7	25	Phosphate buffer	Batch (bubbling)	*27*

found that all tested microorganisms were inactivated by 1.5 to 5 log at 1 to 1.5 ppm of ozone within 15 s. Among these microorganisms, *L. monocytogenes* was the least resistant and *L. mesenteroides* was the most resistant to ozone (Fig. 2).

When compared to vegetative cells, bacterial spores have greater resistance to ozone. Broadwater et al. *(22)* reported that the lethal threshold concentration for *Bacillus cereus* was 0.12 mg/liter while that for *E. coli* and *B. megaterium* was 0.19 mg/liter. The threshold concentration for the spores of *B. cereus* and *B. megaterium* was 2.3 mg/liter. When ozone treatment was combined with other deleterious factors, greater inactivation rates of bacterial spores were observed. Foegeding *(62)* found that acidic pH enhanced the lethality of ozone against the spores of *Bacillus* and *Clostridium.* The author also suggested that the spore coat is a primary protective barrier against ozone. Naitoh *(138, 139)* found that the addition of metallozeolites, ascorbic acid, and isoascorbic acid improved the inactivation of *B. subtilis* spores by ozone treatment at 5 to 50 ppm for 1 to 6 h. Naitoh *(138)* also investigated synergistic sporicidal activities of gaseous ozone and UV irradiation. The author reported that combined treatment reduced the contact time required for the inactivation.

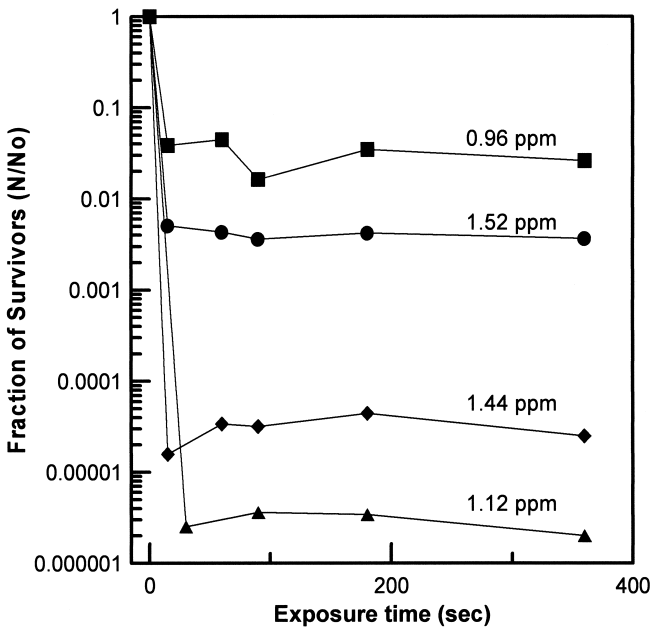

FIGURE 2. *Inactivation of foodborne microorganisms by 0.96 to 1.5 ppm ozone at pH 6.0 and 25°C.* ■, E. coli *O157:H7;* ◆, P. fluorescens*;* ●, L. mesenteroides*;* ▲, L. monocytogenes.

Inhibitory spectrum: fungi. Ozone is an effective fungicidal agent (Table 2). Ewell *(53)* stated that depending on the cleanliness, minimum continuous concentrations of 0.6 to 1.5 ppm ozone were necessary to prevent mold growth on eggs kept at 0.6°C and 90% relative humidity (RH), whereas 2.5 to 3.0 ppm ozone were required to control molds on beef that was stored under similar conditions. According to Farooq and Akhlaque *(56),* ozone also inactivated yeast. The population of *Candida parapsilosis* decreased by 2 log in 1.67 min when the yeast was exposed to 0.23 to 0.26 mg/liter ozone. Counts of *C. tropicalis* decreased by 2 log when the yeast cells were exposed to ozone at 0.02 mg/liter for 20 s or at 1 mg/liter for 5 s *(107).*

Yeasts appear more sensitive than molds to ozone treatments. More than 4.5 log of *C. albicans* and *Zygosaccharomyces bailii* populations were killed instantaneously in ozonated water in a recirculating concurrent reactor, whereas less than 1 log of *Aspergillus niger* spores were killed after a 5-min exposure *(157).* The average ozone output levels in the deionized water was 0.188 mg/liter. Naitoh and Shiga *(145)* found that the threshold of microbicidal activity of aqueous ozone (0.3–0.5 mg/liter) against spores of *Aspergillus, Penicillium,* and *C. paracreus* was 90 to 180, 45 to 60, and 5 to 10 min of exposure, respectively.

TABLE 2. *Inactivation of yeasts by ozone*

Yeast	Inactivation (log_{10})	Treatment time (min)	Concentration (mg/liter)	pH	Temp. (°C)	Medium	Reactor type	Reference
Candida parapsilosis	2.7	1.67	0.23–0.26	7	24	O_3 demand-free water	Continuous flow	*56*
C. tropicalis	2.0	0.30–0.08	0.02–1.0	7.2	20	O_3 demand-free water	Continuous flow	*107*

Yeasts vary in sensitivity to ozone. Naitoh *(140)* treated *Hansenula anomala, Saccharomyces rosei, Pichia farinosa, C. parapsilosis, Kluyveromyces marxianus,* and *Debaryomyces hansenii* var. *hansenii* with gaseous ozone at 4 to 5 ppm for 1 to 5 h at 30 to 60°C and 25 to 90% RH. At lower temperature and 5 h exposure, counts of *C. parapsilosis* and *K. marxianus* decreased more than 1 log; however, counts of the other yeasts did not decrease appreciably. The antimicrobial effect increased with increasing temperature, RH, and treatment time. Ozone increased lag and exponential phases of *H. anomala* and *K. marxianus* by 1.5 to 4 and 1.4 to 6.7 h, respectively.

Inhibitory spectrum: viruses. Ozone is potentially an effective virucidal agent (Table 3). Relatively low concentration of ozone and short contact time are sufficient to inactivate viruses. However, inactivation of viruses in wastewater requires longer contact time and larger ozone concentration than inactivation in ozone demand-free systems because of oxidizable materials present in the medium. Majumdar et al. *(130)* reported a rapid decrease in virus survival at ca. 1 mg/liter initial ozone concentration after a 2-min contact period. Katzenelson et al. *(106)* demonstrated the potent virucidal effect of ozone and suggested that ozone alone or in combination with chlorine be used in treating water and wastewater.

Herbold et al. *(86)* tested the resistance of viruses and bacteria to ozone in steadily flowing water at 20°C and pH 7. The order of resistance was poliovirus 1 < *E. coli* < hepatitis A virus < *Legionella pneumophila* serogroup 6 < *B. subtilis* spores. For the complete inactivation of poliovirus 1 and hepatitis A virus (ca. 10^4 $TCID_{50}$/ml), 0.13 and 0.25 to 0.38 mg/liter ozone was needed, respectively. Emerson et al. *(52)* found that viruses associated with cells or cell fragments are protected from inactivation by ozone at concentrations that readily inactivate purified virus. The authors tested ozone to disinfect human epithelial cells infected with poliovirus (Sabin type) or coxsackievirus A9. In a continuous-flow ozonation system, the cell-associated poliovirus and coxsackievirus samples demonstrated survival at applied ozone dosages of 4.06 and 4.68 mg/liter, respectively for 30 s. Unassociated viruses in the control treatment were inactivated by 0.081 mg/liter for 10 s. Ultrasonic treatment did not increase inactivation of the cell-associated enteric viruses. In a batch reactor, inactivation of cell-associated viruses required 2 min contact with 6.82 mg/liter and ozone residual of 4.7 mg/liter, whereas unassociated viruses were completely inactivated after 5 min with 4.82 mg/liter and ozone residual of 2.18 mg/liter.

Inhibitory spectrum: protozoa. Table 4 lists results of studies on inactivation of some protozoa by ozone. Wickramanayake et al. *(192)* reported the effect of aqueous ozone on the inactivation of cysts of *Naegleria gruberi* and

TABLE 3. *Inactivation of viruses by ozone*

Virus	Inactivation (log_{10})	Treatment time (min)	Concentration (mg/liter)	pH	Temp. (°C)	Medium	Reference
Bacteriophage f2	0.7	10	0.1	7.2	20	Activated sludge effluent	*84*
Bacteriophage f2	>4.3	0.16	0.41	7	20	Water	*18*
Coxsackie virus B5	4.0	2.5	0.4	7.2	20	Sludge effluent	*84*
Coxsackie virus A9	>1.7	0.16	0.035	7	29	Water	*18*
Enteric virus	>1.7	29	Initial 4.1, residual 0.02	7.8	18	Raw wastewater	*100*
Hepatitis A virus	2.7	0.02	0.25	7.2	20	Phosphate buffer	*86*
Human rotavirus	0.7	10	0.31	7.2	20	Sludge effluent	*84*
Poliovirus type 1 (Mahoney)	2.5	1.67	0.23–0.26	7	24	O_3 demand-free water	*56*
Poliovirus type 1 (Mahoney)	1.0	0.53	0.51	7.2	20	Water	*162*
Poliovirus type 1	2.0	10	0.2	7.2	20	Activated sludge effluent	*84*
Vesicular stomatitis	>2.0	0.25		7	25	Phosphate buffer	*27*

TABLE 4. *Inactivation of protozoa by ozone*

Protozoan	Inactivation (log_{10})	Treatment time (min)	Concentration (mg/liter)	pH	Temp. (°C)	Medium	Reactor type	Reference
Cryptosporidium parvum	>1.0	5	1	7	25	O_3 demand-free water	Batch	*118*
Giardia lamblia	2.0	1.1	0.7	7	5	Water	Batch	*192*
G. muris	2.0	2.8	0.5	7	5	Water	Batch	*192*
Naegleria gruberi	2.0	2.1	2.0	7	5	Water	Batch	*192*

Giardia muris. The *N. gruberi* cysts were more resistant to ozone than *G. muris.* A 2-log decrease of population was observed with 0.2 mg/liter ozone at 25°C and pH 7 in 7.5 min for *N. gruberi* compared to 1.05 min for *G. muris.* The intestinal parasite, *Cryptosporidium parvum,* that can cause gastroenteric disease was exposed to ozone that inactivated >90% of the parasite population within 1 min at 1 mg/liter ozone in ozone demand-free water *(118).*

Environmental factors. Although microorganisms inherently vary in sensitivity to ozone, the physiological state (e.g., the stage of growth) and environmental factors affect greatly the degree of inactivation of these microorganisms by ozone. Susceptibility of microorganisms to ozone vary according to the pH of the medium, temperature, humidity, additives (e.g., acids, surfactants, and sugars), and the amount of organic matter surrounding the cells.

Temperature. A decrease in the temperature of an aqueous medium results in increased solubility of ozone. Ozone decomposition, on the other hand, is accelerated with increasing temperature. Herbold et al. *(86)* reported that ozone effectiveness on hepatitis A virus and *E. coli* diminished when the temperature increased from 10°C to 20°C. However, Katzenelson et al. *(106)* indicated that lowering the temperature from 5 to 1°C had a minor effect on the inactivation kinetics of microorganisms.

pH. The stability of aqueous ozone increases by decreasing the pH. Researchers attributed the rapid decomposition of ozone in aqueous solutions with high pH to the catalytic activity of the hydroxyl ion *(1, 87).* Leiguarda et al. *(121)* reported that bactericidal efficiency of ozone on *E. coli* and *C. perfringens* was slightly greater at pH 6.0 than at pH 8.0. Farooq et al. *(57)* noted higher a survival rate of *Mycobacterium fortuitum* during ozone treatment when pH was increased. The authors attributed this increased survival to a smaller ozone residual as the pH of water increased. Foegeding *(62)* studied ozone inactivation of *Bacillus* and *Clostridium* spores at different pH values and found that acidic pH values enhanced the lethality of ozone.

Humidity. Elford and Ende *(51)* used low ozone concentrations and long exposures at variable RHs to disinfect airborne microorganisms. At RH <45%, the germicidal power of ozone was negligible. Inactivation was substantial even at concentrations far below 0.1 mg/liter when high humidities were used. Ewell *(54)* demonstrated that microorganisms were killed more readily by ozone in an atmosphere having a high rather than low RH. The need for moisture in a cell for it to be inactivated by ozone was elucidated by Guerin *(81).* The author indicated that not only were desiccated microorganisms more resistant than hydrated cells to sterilization by ozone, but once desiccated, some cells were difficult to rehydrate sufficiently to be susceptible to ozone sterilization. Guerin concluded that ozone was an effective inhibitor only for nondehydrated microorganisms. Kim and Yousef *(113)* found a similar reaction of ozone in food ingredients containing natural contaminants. They treated a powdered food ingredient, having variable water activities (a_w), with gaseous ozone. When a_w of the ingredient was ca. 0.95, 10^2 to 10^5 CFU/g were inactivated with 200 ppm ozone in an ozone–oxygen mixture. However, similar ozone concentration had no effect on the microbial load of products with a_w less than 0.85. In order to counteract this microbial resistance to ozone, water was added to the powder and the mixture was shaken by an orbital shaker at 25°C overnight. This treatment increased a_w from 0.85 to 0.95 and the total count by 1 log. When the rehydrated product (ca. 8×10^3 CFU/g) was treated with ozone, more than 2 logs were inactivated by 200 ppm and the total count was less than 10^1 CFU/g (the detection limit) when 300 ppm ozone was used.

Ozone demand of the medium. Having a high oxidation potential, ozone reacts with microorganisms fast, resulting in high lethality. Kim *(111)* observed a 2- to 3-log reduction of *P. fluorescens, E. coli* O157:H7, *L. mesenteroides,* and *L. monocytogenes* in <10 s of exposure to <1 ppm ozone in pure cell suspension system. However, ozone also reacts with other particles and compounds if placed in an environment such as food systems that are rich in organic matter. The effectiveness of ozone depends on the amount applied but more so on residual ozone in the medium after demands have been met. Venosa *(186)* pointed out that one of the most serious failures by various investigators has been their inability to distinguish between the concentration of applied ozone and residual ozone necessary for effective disinfection. Therefore, the ozone availability and the decay of ozone during the course of the experiments should be reported, otherwise underestimation of the actual ozone dose used in the experiments to affect the inactivation may follow. Yang and Chen *(197)* reported

that the bactericidal effects of ozone were lower in Ringer solution, 5% NaCl solution, and in the presence of egg albumin than in distilled water. Restaino et al. *(157)* reported that in the presence of organic material, death rates of some gram-positive microorganisms (e.g., *S. aureus* and *L. monocytogenes*), and gram-negatives, *E. coli* and *Salmonella* Typhimurium, in ozonated water were not significantly affected by 20 ppm of soluble starch but were significantly reduced by addition of 20 ppm of bovine serum albumin. Residual ozone in water containing bovine serum albumin was significantly lower than in deionized water and water with soluble starch.

When microorganisms are suspended in an ozone demand-free medium, the only source of ozone demand is the seeded organisms. In water, ozone may react directly with dissolved substances, or it may decompose to form secondary oxidants that immediately react with solutes. These different pathways of reactions lead to different oxidation products, and they are controlled by different types of kinetics *(175)*. The solutes present in water influence appreciably the rate of the radical-type chain reaction leading to the decomposition of ozone. This reaction is promoted by solutes, such as formic acid and methanol, that convert the nonselective hydroxyl ($^{\bullet}OH$) into a superoxide ($^{\bullet}O_2^-$) radical that is a more efficient chain carrier. Such promoters counteract the inhibiting effects of OH radical scavengers that generally terminate the chain reaction. Acetic acid and acetate are known to terminate the reaction by scavenging $^{\bullet}OH$, thus stabilizing ozone in aqueous solutions *(63, 90, 171)*. Schuchmann and Sonntag *(168)* explained ozone effectiveness in reducing the load of organic matter (added D-glucose) in raw water purification. They found that direct mode of reaction by ozone predominated at high glucose concentration; however, the $^{\bullet}OH$ pathway predominated at low glucose concentration, especially at higher pH (e.g., 9.0).

Ozone accessibility to targeted microorganisms. Most microorganisms may not be found in free suspension as discrete particles, specially when they are present in food systems. The association of microorganisms or subcellular components with suspended matter may hamper the accessibility of ozone to microorganisms. Longley et al. *(126)* pointed out that such criteria as degree of mixing and mass transfer must be considered to establish the efficacy of ozone for a particular disinfection application.

Berg et al. *(14)* used the ultrasonic treatment to breakdown clumps of microorganisms and thus increased the antimicrobial effect of ozone dramatically. Burleson et al. *(27)* reported that ozone and sonication resulted in a synergistic effect on the inactivation of viruses and bacteria in secondary effluent. They reasoned that sonication may enhance interphase transport, break up particulate organic material and clusters of bacteria, and produce cavitation to reduce the high surface tension caused by organic matter. However, Kim and Yousef *(112)* could not confirm the effectiveness of sonication during treatment of fresh lettuce with ozone. Sonication may enhance the decomposition of ozone or increase ozone demand by detaching organic materials from the cut surfaces of the shredded lettuce.

OZONE AS AN ALTERNATIVE SANITIZER TO CHLORINE

Merits and drawbacks of chlorine in food processing. Chlorine in various forms, specially hypochlorite salts, has been successfully used to sanitize utensils and equipment in dairy and other food-processing industries. Hypochlorites are considered GRAS substances and thus are permitted in various food application in the United States. Chlorine compounds are effective and inexpensive disinfectants. For example, use of hypochlorite dip or spray is effective in controlling bacterial contamination of fruits and vegetables. In the egg industry, chlorine compounds are used in the wash water to decrease the load of spoilage and pathogenic microorganisms.

Chlorine compounds have a few drawbacks that increasingly limit their use in the food industry. Chlorination may lead to the formation of toxic or carcinogenic chlorinated organic compounds in water *(24, 149)*, and food or on food contact surfaces *(188)*. Collins and Deaner *(41)* reported that chlorine residues >0.1 mg/liter may be excessive with respect to toxicity and that in critical areas of biological significance, it may be necessary to provide dechlorination facilities to reduce chlorine concentration. The recognition of the potential hazard from the presence of carcinogenic trihalomethane compounds (THMs) in drinking water that are formed by the reaction of free chlorine ($HOCl$, OCl^-) with soluble organic compounds prompted legislation that sets the maximum level for total THMs in drinking water at 100 μg/liter *(33)*.

In an effort to control or reduce both the hazardous microorganisms and THM levels in potable water, alternative treatment measures have been proposed. These include pretreatment of water to reduce levels of precursor organic compounds, removal of THMs after chlorination, and application of alternative disinfectants, such as ozone, that do not generate THM *(23)*.

Ozone versus chlorine. Much information attesting to the superiority of ozone over other chemical disinfectants has been accumulated. Gomella *(73)* reported that ozone, compared to chlorine, showed stronger and more rapid antimicrobial action against spores, fecal and pathogenic microorganisms, and viruses, mainly in an environment with a high organic-matter content. Kessel et al. *(108)* showed that free ozone residues of 0.05 to 0.45 mg/liter were sufficient to inactivate poliovirus within 2 min, while free chlorine residues of 0.5 to 1.0 mg/liter at pH 6.0 required 1.5 to 2.0 h for similar degree of virus inactivation. Another study by Scarpino and his colleagues *(167)* also confirmed that ozone was superior to chlorine in the rate of disinfection of poliovirus. With 0.3 mg/liter of disinfectant, ozone reduced virus particle count by 2 logs within 10 s, while chlorine reduced the count by 2 logs in 100 s.

Korich et al. *(118)* reported that chlorine dioxide and ozone were more effective than chlorine and monochloramine against *C. parvum* oocysts. Greater than 90% inacti-

vation of oocysts was achieved with exposure to 1 mg/liter ozone for 5 min. Exposure to 1.3 mg/liter chlorine dioxide yielded 90% inactivation after 1 h, while 80 mg/liter chlorine and 80 mg/liter monochloramine required approximately 90 min for 90% inactivation.

Forsythe and Waldroup *(64)* reported the economic benefits of ozone usage in poultry-processing plants such as reduced water purchase, reduced sewage treatment costs, and savings in electrical energy from recycling ozonated water. With ozone use, for a plant processing 1.3 to 1.5 million broilers a week, weekly savings were expected to be at least $6,000 compared to the use of water without any antimicrobial treatment. In addition to the economic benefits of water recycling, the use of water with fewer chemical residuals will be favorable to the environment.

LIMITATIONS OF OZONE

Reactivity. An often-cited disadvantage of using ozone as a disinfectant is that, unlike chlorine, it is extremely unstable *(132).* It is difficult to predict how ozone reacts in the presence of organic matter. It can oxidize or ionize the compound, or spontaneously decompose to oxygen and free radicals. Mechanisms of decomposition of ozone are complex processes that depend on factors such as the types of radicals formed in solution and various types of organic matter present in the medium that initiate, promote, or inhibit the radical chain reaction. Therefore, it may be difficult to generalize that a particular concentration of ozone at a given rate will always be effective in inhibiting a definite concentration of microorganisms in a food product. After treatment with 1.5 mg/liter ozone, water samples with no detectable residual ozone were found to remain sterile for greater than 1 month *(176).* However, during passage through a pipeline that is 1,200 m long, recontamination and considerable growth of microorganisms were observed. On inoculating water sterilized by ozone with a normal population of water bacteria, growth was more pronounced than in similar experiments with heat-sterilized water of the same origin. This may indicate that the breakdown products of organic water contaminants (e.g., humic acid) produced during ozone treatment are better nutrients for water bacteria than the original organic substances themselves.

Food quality deterioration. Surface oxidation of food may result from excessive use of ozone *(158).* The authors stressed that ozone is not universally beneficial and, in some cases, may promote oxidative spoilage. Fournaud and Lauret *(65)* detected discoloration and undesirable odors in ozone-treated meat. Ozone also changed the surface color of some fruits and vegetables such as peaches *(6),* carrots *(125),* and broccoli florets *(124).* Studies showed that ozone decreased ascorbic acid in broccoli florets *(200)* and thiamin content in wheat flour *(144).* Ozone had a negative effect on the sensory quality of other commodities such as grains *(143),* ground spices *(198)* milk powder *(97),* and fish cake *(37)* due to the lipid oxidation. However, other researchers reported that ozone treatment improved the sensory quality in beef and eggs *(7, 49)* and it did not alter the sensory quality of some fruits and vegetables significantly *(9, 120, 124).* Therefore, alterations in the sensory attributes depend on the chemical composition of food, ozone dose, and treatment condition.

Toxicity. In spite of ozone's pleasant odor at low concentrations, 0.1 mg/liter is objectionable to all normal humans because of irritation to the nose, throat, and eyes *(195).* Scott and Lesher *(170)* reported as little as 0.02 to 0.04 mg/liter can be detected by man, and prolonged exposure to a concentration of 1,000 mg/liter, or greater, can cause death. Thorp *(184)* plotted limiting values for physiological effects of ozone exposure on man. The author indicated that with an hour exposure, symptomatic, irritant, toxic, and irreversible lethal effects can be induced by ozone concentrations of 2, 4, 15, and 95 ppm, respectively. The toxic effects of ozone upon inhalation are manifested in the lungs. A variety of extrapulmonary damage may also result from ozone and its reaction products *(17, 72).*

Davis *(45)* showed that ozone may have a mutagenic effect on *E. coli.* The author suggested that some of the mutagenic effects of UV irradiation is caused by ozone produced by the shorter wavelengths. Hamelin and Chung *(82)* reported an increased mutation rate in *E. coli* exposed to ozone at as low as 0.05 ppm for 5 min. The resulting mutants were sensitive to ozone. Zhurkov et al. *(201),* however, observed a mutagenicity (determined using the Ames test with *Salmonella* strains TA 100 and TA 98) in chlorinated water but not in water subjected to ozone treatment only. Levels of mutagens in chlorinated water could be effectively reduced by subsequent treatment with ozone at 0.5 mg/liter. Mutagenic effects of ozone have also been suggested in plant *(59)* and animal studies *(21, 179).* The supposition that ozone is mutagenic or carcinogenic in man may be questionable if it is based primarily on the information on the biochemical mechanism of ozone toxicity and on in vitro and animal studies.

In practical application of ozone in the food industry, safety-of-use is an important issue. Ozone detection and destruction systems and respirators are needed for the safety of workers in food-processing facilities. In addition, an efficient ozone treatment for the specific application needs to be developed in order to avoid excess of ozone use. Good manufacturing practice and hazard analysis and critical control point systems are also needed to control high ozone-demand materials in food processing. Razumovskii and Zaikov *(155)* indicated the maximum permissible concentration inside buildings is fixed at 0.1 mg/m^3 (0.047 ppm). In the United States, ozone in the work environment is limited to a maximum of 0.1 ppm (vol/vol) on an 8-h/day basis, of a 40-h work week *(32).*

APPLICATIONS OF OZONE IN FOOD PROCESSING

Ozone inactivates microorganisms less effectively when they are on food surfaces than in low ozone-demand liquid media. Inactivation of microflora on food by ozone depends greatly on the nature and composition of food surface, the type of microbial contaminant, and the degree of attachment or association of microorganisms with food.

Meat. The feasibility of using ozone in meat processing has been the focus of several studies. Kaess and Weidemann *(101)* reported that the count of *Pseudomonas* spp. and *C. scottii* on contaminated beef decreased significantly at >2 μg/liter gaseous ozone and the lag phase of *Thamnidium* spp. and *Penicillium* spp. increased, but their growth rate did not change. The color of the muscle surface treated with <0.6 μg/liter ozone did not differ from that of the control treatment. Ozone has been tested in the process of tenderizing meats to control surface microflora (*Pseudomonas* spp., spores, *Salmonellae* spp., *Staphylococcus* spp.). Ozone in a gas mixture at 0.1 mg/liter and RH of 60 to 90% were required in the tenderizing room to inactivate bacteria, but higher concentrations of ozone were required to inhibit molds. Kaess and Weidemann *(102)* also reported that simultaneous use of UV (0.2 μW/cm^2) and ozone (0.5 μg/liter) produced a synergistic inhibitory effect against *Thamnidium* spp. and *Penicillium* spp. This inhibition was manifested by an increase in the lag phase and a decrease in the growth rate. Contrary to these findings, Fournaud and Lauret *(65)* detected little reduction in counts of *Microbacterium thermosphactum, Lactobacillus, P. fluorescens,* and *Leuconostoc* on a beef surface as a result of gaseous ozone treatment (100 ppm) for 30 min. The authors concluded that low activity and side effects such as discoloration and odor development rendered ozone use unacceptable.

Spraying beef brisket fat with hydrogen peroxide (50 g/liter) solution and ozonated water (5 g/liter) was effective in reducing bacterial contamination, when compared to treatments with trisodium phosphate (120 g/liter), acetic acid (20 g/liter) and a commercial sanitizer (3 g/liter) *(74).* Reagan et al. *(156)* conducted a study to compare procedures and interventions for eliminating physical and bacterial contamination from beef carcasses. Rinsing with ozonated water (0.3 to 2.3 mg/liter) reduced aerobic plate counts by 1.3 log CFU/cm^2 that was approximately equivalent to conventional washing in reducing bacterial populations on beef.

Ozone treatment decreased the counts of aerobic mesophiles, coliforms, and sulfite-reducing clostridia in the meat-transport vehicles *(15).* The author reported that ozone treatment also improved the storage quality and decreased counts of mesophilic aerobes and sulfite-reducing anaerobes on meat. Other investigators *(116)* found that ozone at 10 to 20 μg/liter inhibited microbial growth on beef that was kept at 0.4°C and 85 to 90% RH and extended the permissible storage period by 30 to 40%.

Rusch and Kraemer *(164)* used ozone for the treatment of airborne microorganisms on the surface of meat stored at 2.5 to 6°C and 92 to 95% RH. The treatment halted growth of several *Enterobacteriaceae* but not that of *Pseudomonas* spp. When beef carcasses were continuously ozonated (0.03 ppm) at 1.6°C and 95% RH for up to 9 days of ageing, ozone prevented bacterial growth on carcass surfaces; however, it did not increase the retail case life (as judged by odor and appearance) nor did it reduce bacterial growth on retail steaks *(79).* Dondo et al. *(49)* evaluated ozone usage for beef kept in a refrigerator. Ozone stopped the growth of surface contaminants during several days of storage, improved the sensory quality, and decreased the formation of total volatile N compounds. Horvath et al. *(92)* indicated that in the presence of ozone, growth of microflora on meat surfaces decreased at refrigeration temperatures; however, no inhibitory effect was observed if the meat was heavily contaminated.

Poultry. Ozone has been tested for disinfecting hatchery, hatching eggs, poultry chiller water, poultry carcass, and contaminated eggs. Cultures of *Staphylococcus, Streptococcus,* and *Bacillus* species previously isolated from poultry hatcheries and culture collections of *E. coli, P. fluorescens,* and *Salmonella* Typhimurium, *Proteus* species, and *A. fumigatus* were spread-plated on open petri plates and exposed to ozone gas in a prototype laboratory poultry setter *(191).* Ozone treatment (1.5 to 1.65%, wt/wt) decreased microbial populations by >4 to 7 logs for bacteria and >4 logs in the case of fungi. Whistler and Sheldon *(190)* also evaluated ozone as a disinfectant against natural contaminants on hatching eggs. Microbial counts significantly decreased (>2.5 logs) on the shell of eggs that were misted with water and ozonated (ozone in gas mixture was 2.83%, wt/wt) for 2 h. However, hatchability was significantly reduced (26.5 to 37.5%) following ozonation using 3.03% ozone (wt/wt) for 2 h. Bailey et al. *(7)* reported that ozone decreased the aerobic plate counts and *Salmonella* in hatching cabinet air samples by 75 to 99%.

Sheldon and Brown *(172)* evaluated the effects of ozone on the quality of poultry chiller water and broiler carcasses. Carcasses, chilled in tap water containing ozone at 3.0 to 4.5 ppm for 45 min, were consistently lower in microbial count during storage when compared with nontreated ones. Ozonation of chiller water decreased microbial load >2 logs and chemical oxygen demand by ca. 33% and increased light transmission (at 500 nm) without significantly changing the sensory quality of poultry meat. Yang and Chen *(197)* treated broiler parts in ice-cold water with gaseous ozone at 3.88 mg/liter for 20 min and also treated microbial suspension obtained from fresh and spoiled chicken necks with gaseous ozone at 2.48 mg/liter for 5 to 9 min, respectively. According to these authors, the total microbial counts of broiler and microbial suspensions from fresh and spoiled parts decreased 1, 0.6, 3 logs, accordingly. They also noticed that ozone treatment preferentially destroyed gram-negative rods. In another study, ozone was used to disinfect microorganisms on poultry meat *(110).* All microbial contaminants were inactivated when meat was flushed for 50 min with a gas mixture containing ozone flowing at 1,500 ppm/min.

Rudavskaya and Tishchenko *(163)* evaluated the quality and keeping characteristics of retail eggs after ozonation. Eggs were treated with ozone gas (10 to 12 μg/liter air) for 6 h and then stored for 6 months at −1°C with 86% RH and 29°C with 75% RH. Eggs were analyzed for sensory quality, changes in acid, peroxide and thiobarbituric acid values of the yolk, white and yolk indices, and variations in quality grading. All quality parameters had better values in the ozone-treated samples than in the controls, and the

lower storage temperature had an additional beneficial effect on quality. Krivopishin et al. *(119)* suggested a method for preservation of eggs using ozone. Eggs were dipped in paraffin wax at 40 to 45°C and treated for 10 to 30 min in air containing 1 to 3 mg/liter ozone. Cox et al. *(43)* patented a hyperpasteurization process that involves treatment of washed egg shell with heat (59.4°C) and ozone in a vacuum chamber. The treated eggs have extended shelf-life and reduced microbial load.

Fruits and vegetables. Ozone treatments increased the shelf life of some fruits. Bazarova *(12)* stored apples in a specially constructed stainless steel chamber at 0 to 1°C and 90 to 95% RH with ozone gas being admitted daily for 4 h at 5 to 6 μg/liter. The author concluded that ozone treatment reduced weight loss and spoilage incidence in apples. Ozone at 0.1 to 0.3 ppm in atmosphere during blackberry storage suppressed fungal development for 12 days at 2°C and did not cause observable injury or defects *(11).* Grapes exposed for 20 min to ozone (8 mg/liter) had considerably reduced counts of bacteria, fungi, and yeasts *(166).* Fungal decay following cold storage of the grapes was reduced and shelf life increased by the ozone treatment. Horvath et al. *(92)* attributed the increase of the shelf life of apples and oranges to the oxidation of ethylene and to the removal of other metabolic products by ozone. However, inactivation of spoilage microorganisms on fruits, without a doubt, contributed to this shelf life extension.

In vegetables, the advantages of ozone were similar to those experienced in fruit processing. Onions and potatoes were stored in wooden chambers covered with polyethylene film in which ozone (0.2 μg/liter) was produced for 8 h/day on 5 days/week *(55).* Ozone treatment decreased chemiluminescence, oxygen uptake, catalase, and peroxidase activities and had a marked inhibitory effect on the growth of surface microorganisms. Losses due to spoilage at the end of storage were 1 and 0.8%, respectively, for treated onions and potatoes versus 9.7 and 6.7% for controls. Baranovskaya et al. *(9)* used ozone in the industrial storage of potatoes, onions, and sugar beets. They maintained ozone concentration at 3 mg/liter with temperature within 6 to 14°C and RH at 93 to 97%. Their analysis showed that bacteria and mold counts were very low for treated samples, whereas chemical composition and sensory quality did not change appreciably. Ozone was presented to be an alternative to chlorpropham(isopropyl-*N*-[3-chlorophenyl]carbamate) as a sprout control agent for Russet Burbank potatoes in Canada *(151).*

Kim et al. *(111)* treated shredded lettuce with ozone under different mechanical actions such as sonication, stirring, and stomaching. Bubbling ozone gas (4.9%, vol/vol; 0.5 liter/min) in a lettuce–water mixture decreased the natural microbial load by 1.5 to 1.9 logs in 5 min. These authors concluded that bubbling gaseous ozone was the most effective ozonation method. For efficient ozone delivery to microorganisms on lettuce, ozone bubbling should be combined with high-speed stir. Carrots, inoculated with pathogenic fungi, *Botrytis cinerea* Pers. and *Sclerotinia sclerotiorum* de Bary, were exposed to a gas mixture containing 0 to 60 mg/liter ozone at a flow rate of 0.5 liter/min for 8 h daily for 28 days *(125).* A 50% reduction in daily growth rates for both fungi was obtained at 60 mg/liter ozone. Carrot respiration rate, electrolyte leakage, and total color differences increased with ozone concentration. Ozone-treated carrots were lighter (higher L values) and less intense (lower chroma values) in color than control carrots. Williams et al. *(193)* designed a twin pass pressurized mass transfer system to improve ozone solubility in carrot wash water and obtained considerable reduction in microbial count. Ozonation was applied to pilot-scale treatment of carrot wash water *(194).* At an ozone injection rate of ca. 5 g/liter/h, total and fecal coliform counts decreased >3 log after 30 min ozonation.

Naitoh and Shiga *(146)* reported that simultaneous treatment with an ozone–air mixture (0.02 to 0.2 ppm) and ozone water (0.3 to 0.5 ppm) decreased total microbial count and elongation of hypocotyls of bean sprouts (black matpe and alfalfa). Catalase and superoxide dismutase activities increased significantly with ozone treatment during germination.

The effects of treating kimchi ingredients (cabbage, hot pepper powder, garlic, ginger, green onion, and leak) with ozone gas (6 mg/liter/s for 60 min) on the vitamin content, bacterial count, and sensory properties of this product were investigated by Kim et al. *(114).* Ozone treatment eliminated 80 to 90% of the total bacterial population in garlic and ginger and improved sensory properties of kimchi. Black peppercorns, contaminated with *Salmonella* spp., *S. aureus, B. cereus, Penicillium* spp., or *Aspergillus* spp., were immersed in water and sparged with gaseous ozone (6.7 mg/liter) for 10 min at a flow rate of 6 liter/min *(199).* Ozone treatment decreased the microbial counts by 3 to 4 logs.

Several patents for preservation of fruit and vegetables by ozone technology are currently available. Cantelli *(28)* developed a method based on holding the produce in a sealed container while maintaining an electrical discharge that forms ozone and nitrogen oxides, at concentrations of ca. 0.05 ppm and 0.5 ppm, respectively. Karg *(105)* obtained a patent for sterilization of heavily contaminated foods such as herbs, spices, fruits, and vegetables by ozone treatment. His process comprises an initial conditioning phase, treatment of gas mixture containing ozone, and elimination of residual ozone. Mitsuda et al. *(134)* patented a method to sterilize foods such as fish, fruits, vegetables, and beef, in a processing room, packing receptacles, or a refrigerator using a gas mixture that includes O_3, CO_2, and/or N_2. Hurst *(95)* developed a method for sanitizing food products by immersion of the product in a bath supplied with a continuous stream of ozone-containing bubbles. Rosenthal *(161)* obtained a patent for sanitizing fruits with an apparatus consisting of UV, infrared radiation, and ozone water.

Dry foods. *Bacillus* and *Micrococcus* are dominant bacterial genera of cereal grains, peas, beans, and spices. Counts of these microorganisms decreased 1 to 3 logs by <50 mg/liter ozone *(143).* Naitoh et al. *(142, 143)* studied

the effects of ozone concentration (0.5 to 50 mg/liter), exposure time (1 to 6 h), and temperature (5 to 50°C) on several cereal grains, cereal grain powders, peas, beans, and whole spices. With few exceptions, longer exposure time and lower temperature resulted in higher microbicidal activity in these dry foods. The authors found that oxidation of lipids in these commodities rarely occurred at <5 ppm but was considerable at higher concentrations. Naitoh et al. *(144)* reported the treatment of wheat flour with 0.5 to 50 ppm ozone for 6 h. This treatment inhibited microbial growth in namamen product and increased storage life two- to fivefold. During the storage time, thiamine content decreased 4 to 17%, but sensory quality of namamen did not change. In a microbial decontamination study of spices by gaseous ozone, several samples showed only a slight (<1 log) microbial inactivation with 30 to 145 mg/liter residual ozone but white pepper showed a 4.4-log reduction *(198).* Ozonation also decreased essential oil content and had a negative effect on the sensory quality of some spices.

Ozone was tested on garlic during long-term cold storage *(69).* Ozone increased the yield of stored garlic by 3.7% and decreased damage to the product by *Penicillium.* A flow reactor was used to study the feasibility of using ozone to oxidize odors produced during dehydration of onions and garlic *(131).* Ozone treatment (5 to 20 ppm for 30 s) destroyed 60 to 90% of the individual gaseous components from onions and garlic oils.

Ground black pepper samples, containing various moisture levels, were sparged with an ozone–air mixture (6.7 mg/liter) for up to 6 h *(199).* Total aerobic and anaerobic bacterial counts of treated samples decreased by 3 to 6 logs depending on the moisture content. Higher moisture content led to a greater reduction in the microbial load. Ozone treatment of ground black pepper resulted in the oxidation of certain volatile oil constituents, while the treatment had no significant effect on volatile oil constituents of whole peppercorns.

Ozone was applied in heating peanut meal to destroy aflatoxins or to greatly reduce their levels *(48).* Weight gains for ducklings and rats receiving treated meals were essentially comparable to control animals, however, treated meals had reduced protein efficiency ratios. Rayner et al. *(154)* reported that ozone reduced aflatoxin in cottonseed meal and peanut meal. Contaminated cottonseed and peanut meals were hydrated and brought into contact with ozone at 75 to 100°C to achieve substantial lowering of the aflatoxin content. With 15 mg/liter for 30 min, ozone effectively decreased the *A. flavus* population and its aflatoxin in dried soup *(150).* The destruction and detoxification of aflatoxins B_1, G_1, B_2, and G_2 (50 μg/ml in 4% dimethyl sulfoxide) with ozone were confirmed by Maeba et al. *(129).* Aflatoxins B_1 and G_1 were degraded with 1.1 mg/liter ozone within 5 min; however, B_2 and G_2 required 34.3 mg/liter ozone and 50 to 60 min treatment time for comparable degradation. Chatterjee and Mukherjee *(36)* studied the impact of ozone on the immunity-impairing activity of aflatoxin B_1. The phagocytosis-suppressing activity of aflatoxin B_1 was destroyed with gaseous ozone treatment (1.2 mg/liter) for 6 min at a flow rate of 40 ml/min.

Cheese. Ozone concentrations of 0.1 and 10 μg/liter in the atmosphere of a cheese-ripening room inactivated 80 to 90 and 99%, respectively, of mold spores without affecting the sensory qualities of cheeses *(173).* Batches of Rossiiskii, Poshekhonskii, Kostroma, and Swiss-type cheeses were stored at 2 to 4°C and 85 to 90% RH with ozone generated in the atmosphere of the storage area *(68).* Researchers found that periodical ozonization for at least 4 h at 2- to 3-day intervals with 5 to 7 μg/liter ozone in air prevented growth of molds on cheeses and packaging materials for 4 months without adversely affecting chemical and sensory properties of the cheese. Control cheese exhibited mold growth as early as 1 month. Horvath et al. *(92)* noted that storage life of cheese increased to 11 weeks by the application of ozone at low concentrations (0.02 mg/liter) during the ripening period. Other experiments conducted on cheddar cheese also indicated that the oxidizing action of ozone removes odors otherwise present in storage rooms. Shiler et al. *(174)* described a method of ozonation for ripening and storing cheese to inactivate contaminating microflora but to avoid damage to cheese-packaging materials and to improve hygiene. For optimum results, ozonization was carried out for 1 to 3 h/day at an ozone concentration in the air of 0.08 to 0.1 μg/liter with intervals of 2 to 12 h, and every 10 to 30 days the chambers were treated with ozone at a concentration of 8 to 12 μg/liter for 2 to 4 h.

Fish. In the fishery industry, ozone was tested to disinfect fishery products and to improve sensory qualities. Haraguchi et al. *(83)* studied the preserving effect of ozone on fresh jack mackerel (*Trachurus trachurus*) and shimaaji (*Caranx mertensi*). Treatment of the skin of the gutted fish with 3% NaCl solution containing 0.6 ppm of ozone for 30 to 60 min decreased the viable bacterial count by 2 to 3 logs. The storage life of the fish increased 20 to 60% when the ozone treatment was applied every 2 days. Chen et al. *(37)* studied ozone for in-plant sterilization of frozen fishery products. They found that ozone was effective in distilled water and 3% NaCl solution for the inactivation of microorganisms such as *Vibrio cholera, E. coli, Salmonella* Typhimurium, *V. parahaemolyticus,* and *S. aureus.* Ozone treatment of shrimp decreased *E. coli* count by 98.5%. Coudrains and Starck *(42)* applied 10 to 15 mg/liter gaseous ozone in the air for 4 to 6 min to remove odor and color from fish flesh. Dondo et al. *(49)* reported that ozone decreased surface contaminants of fish during several days of refrigerated storage. Ozone treatment improved the sensory quality of fish by decreasing the formation of trimethylamine. A beneficial decoloration effect of horse mackerel (*T. japonicus*) mince resulted from washing with ozonated water for 10 to 20 min *(38).* However, a marked decrease in pH and undesirable gel strength of mince, as well as oxidation of the fish oil, occurred during this ozone treatment. Ozone promoted detachment of the surface slime of redfish aboard fishing vessels and ozonation during transport reduced bacterial count and extended the shelf life of the fish by ca. 1.5 days *(115).* Simulation trials in the laboratory indicated that bacterial counts were higher on fish

held in ozonated water than on control fish. The author attributed this difference to the lower freshness of redfish used in the laboratory. Therefore, it was recommended that fish should be treated with ozone when it is fresh. Ozone was tested to improve the washing process that is applied during the manufacture of dark-fleshed fish surimi *(39).* The investigators found that ozone washing treatment minimized the washing time and improved color; however, undesirable gel strength and a decrease in the pH of the minces were observed.

Water and fluid food. Sander *(165)* developed an ozone treatment for fruit juices and liquid dairy products that minimizes possible quality deterioration. Rojek et al. *(159)* attempted to use pressurized ozone to decrease the microbial population of skim milk. In this study, ozone gas concentration was 5 to 35 mg/liter and treatment time was 5 to 25 min. Their results showed that pressurized ozone was effective in decreasing psychrotrophic counts by 2.4 logs. Treatment of whey and apple juice also produced favorable microbial reduction. Greene et al. *(77)* proved effectiveness of ozone against biofilms of milk spoilage bacteria, such as *P. fluorescens* and *Alcaligenes faecalis,* on stainless steel plates. Greater than 99% of the population was eliminated by ozone treatment at 0.5 ppm for 10 min.

Franz and Gagnaux *(66)* investigated an ozone treatment to sterilize contaminated spring water for use in the food industry. They found that coliforms and spore-forming bacteria were inactivated during 8 min treatment at 0.1 to 0.2 mg/liter and 1.6 to 3.2 mg/liter ozone, respectively. However, in an industrial installation, only 80% sterilization was achieved within 14 min and ozone concentrations of 1.12 to 2.18 mg/liter. Ozone consumption increased with increases in suspended matter and the pH. They also reported that preliminary flocculation decreased ozone consumption and produced completely germ-free water. Possible applications of ozone in the brewery industry were suggested *(183).* These include yeast washing, selective removal of bacterial contaminants and final rinses of bottles, cans, fillers, pipelines, and tanks.

Ozone is considered one of the means of ensuring water quality in the beverage industry *(67).* Hargesheimer and Watson *(85)* reported that ozone altered the fishy odor associated with some phytoplankton blooms in drinking water sources to an undesirable plastic-like odor. They suggested a combination of granulated activated carbon with ozonation for removal of particulates, color, taste, and odor compounds. The water for ice manufacture may also be sterilized with ozone *(4).* Ozone was used for ageing a fermented product, such as a distilled liquor *(122).*

Process water and effluents. Woerner et al. *(196)* examined direct ozonization to disinfect protein-containing fluid synthetic media, household effluent, and slaughterhouse effluent. They found that 5 to 10 mg/liter gaseous ozone was adequate to eliminate bacteria according to the degree of contamination. *Salmonellae* were eliminated after a contact time of 7 min and anthrax spores after 30 min. While describing possible methods for sterilization of slaughterhouse effluents, Boehm *(16)* suggested ozone treatment as the best chemical method. Hurst *(94)* patented a method by which ozone is bubbled through the food process water to remove fat, bacteria, solids, and other impurities before recycling this water. Postprocess spoilage of canned food decreased by using ozonated water for cooling cans *(98).* Loorits et al. *(127)* explored the possibility of using ozone for oxidizing major milk components. Ozone reduced the fat content in condensates (80 to 230 mg/liter) by 96 to 98% and completely eliminated turbidity. The authors concluded that ozone treatment could be applied to the purification of lightly polluted dairy effluent for subsequent reuse in water supply systems. The chemical oxidation of olive mill effluents by ozone was developed to reduce chemical oxygen demand, aromatic content, and phenolic content *(13).*

By-products. Egg shells were broken into small pieces and subjected to the action of ozone. After the shell became fine powder, it was subjected to ozone again to make it free of bacteria *(135).* Ozone was used to destroy the porphyrin structure in swine hemoglobin and to prepare decolorized protein products *(34).* The same authors *(35)* reported that amino acid analysis and sulfhydryl group determination demonstrated that cysteine and disulfide bonds were completely destroyed during the decolorization process.

Processing plant. Disinfection of air is an important part of clean room technology in the food industry. Holah et al. *(91)* evaluated different air disinfection systems and found that ozone was effective and reproducible in its effect on airborne microorganisms. Ozone also can be applied for preventing secondary contamination during bread manufacturing *(177).* The interior environment in a factory that manufactures plastic films was exposed to 0.02 to 0.16 ppm ozone for 10 h per day and 1 to 1.5 years *(141).* Aerial contaminants such as *Bacillus* spp. and *Micrococcus* spp. in the plastic film processes were reduced. Chun et al. *(40)* developed a UV air cleaner for the sterilization and deodorization of the air in refrigerators. The authors reported that ozone production reached 0.082 ppm in the holding section at 25°C and 0.06 ppm at 3°C. The bactericidal action of activated oxygen (O_2, O_3, and O) destroyed or reduced organisms on food preparation surfaces and inhibited development of cold-tolerant bacteria and pseudomonads on foods *(3).* Decupper *(46)* obtained a patent to use ozone and UV sterilization unit for cold storage of foods.

Greene et al. *(78)* tested the resistance of standard-molded, one-piece O-ring food-processing plant gaskets (36.1 mm) made of seven different substances (Buna N, white Buna N, ethylene propylene diene monomer, polyethylene, silicone rubber, Teflon, and steam-resistant Viton) against chlorine sanitizer and ozonated water (0.4 to 0.5 ppm). Ozone treatment affected the tensile strength of EPDM and Viton but not significantly more than chlorine treatment. The elasticity of an ozone-treated Teflon gasket was significantly different from chlorine-treated ones.

Miscellaneous applications. Karg *(104)* developed an ozone treatment process using gaseous or supercritical ozone for nonfood products, plants, herbs, or spices. Green

tea was converted into black tea by heating an aqueous solution of green tea solids at pH >6.0 in the presence of ozone *(76).* Ozone was introduced to prevent bean expansion in steeping raw coffee beans and also used to treat roasted beans *(189).* Ozone-treated beans did not contain H_2S and had a bright and smooth surface. A process for preservation and/or sterilization of feeds and tobacco products is also based on fumigation with ozone *(26).* Ozone sterilization of food-packaging materials may cause oxidation of antioxidants used in such packaging *(178).* Volodin and Shiler *(187)* tested the applicability of different plastic films for cheese packaging. They reported that thin films such as VIM-K, Novallen-K, and Saran (12 μm) were suitable for surface-sterilization treatment of packaged cheese with ozone because of their ozone permeability; however, thick films such as VIM-D (260 to 470 μm), Novallen-D (300 to 480 μm), Saran (37 μm), Hostaphan (90 μm), and KOD 115 (76 μm) were not permeable to ozone. Ong et al. *(148)* examined the effectiveness of chlorinated and ozonated water dips in the dissipation of pesticide (azinophos-methyl, captan, and formetanate hydrochloride) in solution, and on fresh and processed apples. Both treatments decreased pesticides in solution and on apples. However, the ozone wash at 0.25 mg/liter was not as effective as the chorine washes at 50 and 500 mg/liter because of the low ozone concentration and the high organic content of the wash water.

CONCLUSION

Previous studies indicate that ozone can be used as a safe and effective antimicrobial agent in many food applications. When compared with chlorine and other disinfectants, lower concentrations of ozone and shorter contact times are sufficient in controlling or reducing microbial population. Ozone is also more effective than other disinfectants against resistant organisms such as amoebic cysts and viruses. Exposure to ozone during processing or storage extends the shelf life of certain products such as fruits and vegetables while preserving its sensory attributes. Ozone does not produce significant toxic residues in the environment after the treatment.

More studies are required to define inherent factors that contribute to the resistance of some microorganisms to ozone. Such studies will help define the most-resistant microorganism to ozone for use as indicators of sanitization by this agent. Increasing the effectiveness of ozonation processes while optimizing ozone use is an urgent issue for successful applications in food processing. With acquired knowledge and experience, operating specifications and protocols can be developed to use ozone at the most efficient and safe level.

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