OZONE AND ITS CURRENT AND FUTURE APPLICATION IN THE FOOD INDUSTRY

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ABSTRACT

The food industry is interested considerably in using ozone to enhance the shelf-life and safety of food products and in exploring new applications of the sanitizer. This interest was recently accompanied by a US governmental approval of ozone for the safe use, in gaseous and aqueous phases, as an antimicrobial agent on food, including meat and poultry. Ozone has a strong microbicidal action against bacteria, fungi, parasites and viruses when these microorganisms are present in low ozone-demand media. Readily available organic constituents in food, however, compete with microorganisms for applied ozone and thus efficacy of the treatment is minimized. Ozone is suitable for washing and sanitizing solid food with intact and smooth surfaces (e.g., fruits and vegetables) and ozone-sanitized fresh produce has recently been introduced in the US market. Use of ozone to sanitize equipment, packaging materials, and processing environment is currently investigated. Efforts to decontaminate bean sprouts and remove biofilm with ozone have not been successful. The antimicrobial efficacy can be enhanced considerably when ozonation is combined with other chemical (e.g., hydrogen peroxide) or physical (e.g., ultraviolet radiation) treatments. Mechanical action is also needed as a means to dislodge microorganisms from the surface of food and expose them to the action of the sanitizer. The food industry also is interested in using ozone to decontaminate processing water and decrease its chemical and biological oxygen demand. This application improves the reusability of processing water and allows for environment-friendly processing operations.

I. INTRODUCTION

Ozone has been applied industrially for many years, mostly in water treatments, because of its high oxidizing power and superior antimicrobial properties. Use of ozone in the food industry, however, has been limited mainly to shelf-life extension of commodities during storage. Recently,

there has been a renewed interest in ozone and its application in food processing. Application of ozone for decontamination of poultry chiller water seems promising (Sheldon and Chang, 1987; Waldroup *et al.*, 1993; Diaz and Law, 1999) and washing fruits and vegetables with ozone is gradually gaining acceptance. Novel application of this powerful sanitizer will be addressed in this chapter.

Current sanitization technologies are crucial to maintaining the quality and enhancing the safety of fresh agricultural commodities. These technologies, however, have many drawbacks and some treated products are potentially hazardous to consumers. Safety of produce, which is commonly treated with chlorine (or occasionally consumed unsanitized), is currently questionable because of frequent disease outbreaks associated with these products. Pathogens resistant to preservation factors, such as acid-tolerant *Salmonella* spp., *Listeria monocytogenes* and *Escherichia coli* O157:H7, have emerged as a serious threat to the fresh produce industry (Beuchat, 1995; Odumeru *et al.*, 1997; NACMCF, 1999). Salmonellosis outbreaks have been associated with pre-cut watermelons and cantaloupes and fresh tomatoes contaminated with *S.* Javiana, *S.* Montevideo and *S.* Poona (Gayler *et al.*, 1955; Ries *et al.*, 1990; Wood *et al.*, 1991; CDC, 1993; *LA Times*, 2001). Water used to wash the tomatoes was implicated as the source of contamination. Outbreaks linked to consumption of unpasteurized apple juice and cider (Besser *et al.*, 1993; CDC, 1996b) also may be attributed to the failure in sanitizing apples properly. Oocysts of *Cryptosporidium parvum*, a zoonotic protozoan parasite, have been detected in unpasteurized cider which caused a disease outbreak (Millard *et al.*, 1994). In 1996, a large epidemic in the United States and Canada involving another protozoan parasite, *Cyclospora cayetanensis*, was epidemiologically linked to raspberries that were imported from Guatemala (CDC, 1996a). Use of nonpotable water for spraying the plant with fungicide was suggested as the source of the pathogen.

In addition to fresh produce, the meat industry can also benefit greatly from new developments in sanitization technology. Meat products have caused numerous foodborne disease outbreaks. *Listeria monocytogenes* has been associated with ready-to-eat meat products resulting in multistate outbreaks of listeriosis in 1998 and 2000 (CDC, 1998, 2000). *Escherichia coli* O157: H7 is traditionally linked to beef products (CDC, 1996c, 1997). This pathogen has caused several disease outbreaks due to consumption of undercooked meat, and the bacterium most likely entered the processing chain on contaminated carcasses.

It is obvious that effective, reliable, economical and industry-relevant alternative sanitization methods are needed. At present, chemical disinfectants such as chlorine and hypochlorites are commonly used as

sanitizing agents in the food industry. Treatments with 50–100 ppm free chlorine solutions reduce initial contamination of vegetables (Carlin *et al.*, 1995), but these levels of the sanitizer may lead to discoloration and production of off-flavors in fresh produce (Hurst and Schuler, 1992). Additionally, chlorination of food could lead to the formation of toxic and carcinogenic chlorinated compounds (Brungs, 1973; Page *et al.*, 1976; Kirk and Mitchell, 1980).

Ozone is an effective, chlorine alternative, sanitizer with superior antimicrobial properties (Kessel *et al.*, 1943; Ito and Seeger, 1980; Korich *et al.*, 1990). It is capable of inactivating bacteria, bacterial spores, molds, yeasts, protozoan cysts and viruses at relatively low concentration and in short exposure time when applied to pure cell suspensions (Giese and Christenser, 1954; Scott and Lesher, 1963; Kim, 1998). Ozone has been tested on nearly every type of food during storage and processing to improve the safety and to extend the shelf-life of these products. The ability of ozone to inactivate contaminant microflora on food is variable; in some instances, however, ozone decreased food microflora more than 5 log units (Yousef and Rodriguez-Romo, 2001). Ozone not only inactivates microbial contaminants, but is also potentially useful in decreasing the level of pesticides, such as azinphosmethyl, captan, formethanate-HCl and ethylenethiourea, on fresh produce (Ong *et al.*, 1996; Hwang, 1999). The chemical oxygen demand (COD) and biological oxygen demand (BOD) of water used in washing and processing of foods can be decreased appreciably by ozonation (Sheldon and Brown, 1986). Thus, use of ozone minimizes the accumulation of inorganic waste in the environment (Horvath *et al.*, 1985). Moreover, rapid decomposition to oxygen and lack of toxic residues make ozone a favorable environment-friendly sanitizer.

Ozone is currently used in many countries and its use in food processing has been approved recently in the United States (Federal Register, 2001). Additionally, ozone-treated produce has just been introduced in the United States market. This chapter addresses current applications of ozone in the food industry, problems that were recently encountered in attempts to apply ozone in food processing, and some probable and challenging future applications. Some of the application problems originated from lack of basic knowledge on sanitization and others are ozone-specific. Recent ozone findings are presented with emphasis on improving the safety of fresh produce.

II. OZONE CHEMISTRY AND PHYSICS: AN OVERVIEW

Ozone is formed in the stratosphere (15–35 km altitude) by the action of short ultraviolet (UV) solar radiation (< 240 nm) on molecular oxygen and

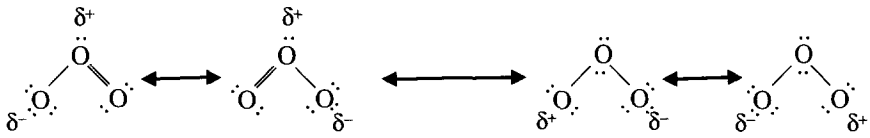

FIG. 1. Resonance structures in ozone molecules (Trambarulo *et al.*, 1953).

TABLE I
OXIDATION–REDUCTION POTENTIAL (VOLTS) OF DIFFERENT CHEMICAL OXIDANTS

Agent	Molecular formula	Oxidation–reduction potential
Fluorine	F_2	2.87
Ozone	O_3	2.07
Hydrogen peroxide	H_2O_2	1.78
Potassium permanganate	$KMnO_4$	1.70
Hydrobromous acid	HOBr	1.59
Hypochlorous acid	HOCl	1.49
Chlorine	Cl_2	1.36
Chlorine dioxide	ClO_2	1.27
Oxygen	O_2	1.23
Chromic acid	H_2CrO_4	1.21
Bromine	Br_2	1.09
Nitric acid	HNO_3	0.94
Iodine	I_2	0.54

a small portion of ozone is transported to the troposphere (< 15 km altitude). About 10% of the atmospheric ozone is present in the troposphere but a very small concentration of ozone occurs naturally at the Earth's surface (Wojtowicz, 1996). Large amounts of the gas can be synthesized by generators for industrial use. Ozone is a triatomic molecule (O_3) that is considered to be an allotropic modification of oxygen. It has a relative molecular mass of 48 and its molecular structure is a resonance hybrid of the four canonical forms having delocalized bonding (Figure 1). Pure ozone is a pale blue gas and bluish liquid with pungent and characteristic odor. Ozone exists in the gaseous state at room and refrigeration temperature and it is partially soluble in water. Ozone has an oxidation–reduction potential of 2.07 V (Brady and Humiston, 1978), which makes it the strongest oxidant currently available for food applications (Table I). The density of ozone in the gaseous state is 2.14 g L^{-1} at 0°C and 101.3 kPa (Wojtowicz, 1996), which is greater than that of air (1.28 g L^{-1}) under similar conditions.

A. OZONE SOLUBILITY

Ozone is partially soluble in water and its solubility depends on several physical parameters. By Henry' law, ozone solubility in liquid is directly proportional to the pressure that the gas exerts above the liquid (Bablon *et al.*, 1991). The most important parameter affecting the solubility of ozone is probably water temperature. Based on Henry–Dalton constants, the solubility of ozone in water is higher at lower temperatures. Watson (1943) reported that the solubility ratio (ozone concentration in water phase/ ozone concentration in gas phase) was 0.26 at 20°C. Meddows-Taylor (1947) reported a solubility ratio of ~ 0.4 at 20°C. According to Horvath *et al.* (1985), the solubility ratio of ozone in water was 0.31–1.13, depending on the water temperature. Our data showed that solubility ratio varied with the source of water used to solubilize ozone. The solubility ratio was 0.16 for distilled water at ~ 22°C, after ozone was bubbled in the water at 29.4 mL min^{-1} for 18 min (Kim, 1998). When ozone was sparged for 18 min into tap (from two different sources) and deionized water under similar conditions, the solubility of ozone was 38, 56 and 95% of that for distilled water, respectively (Figure 2). Variations in published solubility data may be attributed to differences in the reactor design, gas flow rate and analytical method used to quantitate ozone.

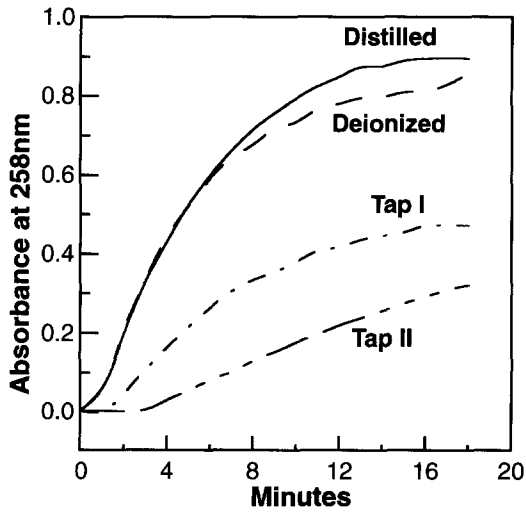

FIG. 2. Treatment of water from different sources with ozone gas (~ 2.5%, v/v) at a flow rate of 29.4 mL min^{-1} (Kim, 1998). Tap I, tap water in the research laboratory; Tap II, drinking fountain water.

High pH interferes with the solubility of molecular ozone. As the pH of ozone solutions increases, the rate of decomposition of molecular ozone into hydroxyl radical also increases (Adler and Hill, 1950; Hewes and Davison, 1973). Researchers attributed the rapid decomposition of ozone in aqueous solutions with high pH to the catalytic activity of the hydroxyl ion. Farooq et al. (1977) noted a greater survival rate of *Mycobacterium fortuitum* during ozone treatment when the pH of the treatment medium was increased. The authors attributed this increased survival to a smaller ozone residual concentration as the pH of water increased. Hydroxide ions are consumed in initiating the ozone decomposition process in water; therefore, ozonation of a solution can decrease its pH value. Sheldon and Chang (1987) found that the pH of poultry-processing water, containing a high organic load, decreased from 6.9 to 5.6 after 50 min ozonation.

B. OZONE STABILITY

Ozone is more stable in the gaseous than in the aqueous phase (Stumm, 1958). Stability of dissolved ozone (measured as half-life) is affected by its concentration, pH of the aqueous medium, exposure to UV radiation, presence of radical scavengers (Tomiyasu *et al.*, 1985; Kim, 1998), application of turbulence (Shechter, 1973), temperature (Sease, 1976), and presence of organic matter and metal ions (Horvath *et al.*, 1985). Decomposition of ozone in water does not always follow the first-order rate law (Gurol and Singer, 1982; Tomiyasu *et al.*, 1985; Yurteri and Gurol, 1988). The stability of ozone in water decreases when the pH of the medium increases. High pH is also believed to interfere with the solubility of molecular ozone (Roth and Sullivan, 1981; Ouederni *et al.*, 1987). Kim (1998) found that ozone decomposes rapidly in phosphate buffer when the pH is greater than 8.0 (Figure 3). The researcher bubbled ozone in different types of water and phosphate buffer (pH 7.0) at 25°C, to attain 1.6–2.5 ppm, and monitored ozone decomposition spectrophotometrically. The rate of ozone decomposition was greater in tap water and buffer than it was in distilled, deionized and HPLC-grade water (Figure 4). Ozone stability in the water is greatly influenced by the presence of contaminants, particularly metal ions. According to Bablon *et al.* (1991), however, ionic strength resulting from mineralization of drinking water ($< 1000\ mg\ L^{-1}$ total dissolved solids) does not affect the solubility of ozone appreciably.

Temperature plays an important role in the stability of ozone in solutions. The half-life of ozone in the gaseous state is approximately 12 h at room temperature and in pure, clean water (pH 7–8) it is 20–30 min (Graham, 1997). The stability of ozone in solutions also depends greatly on the amount of ozone-demand material in the water. Rosenthal (1974)

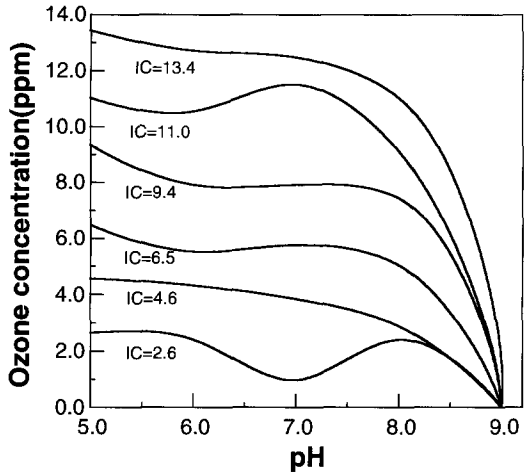

FIG. 3. Ozone stability in phosphate buffer having different pH values (Kim, 1998). IC, calculated initial ozone concentration in the ozone–buffer mixture.

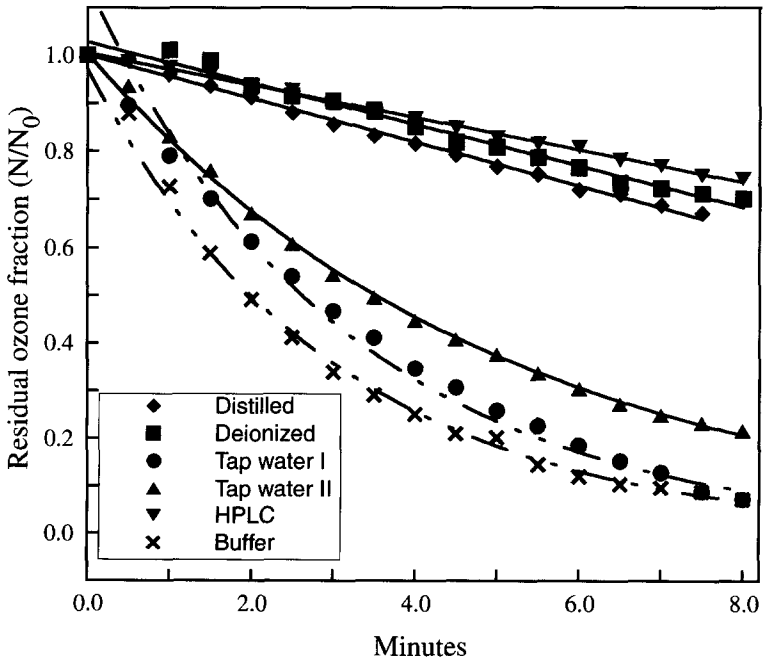

FIG. 4. Ozone decomposition in water from different sources (Kim, 1998). N_0, ozone concentration initially; N, residual ozone concentration.

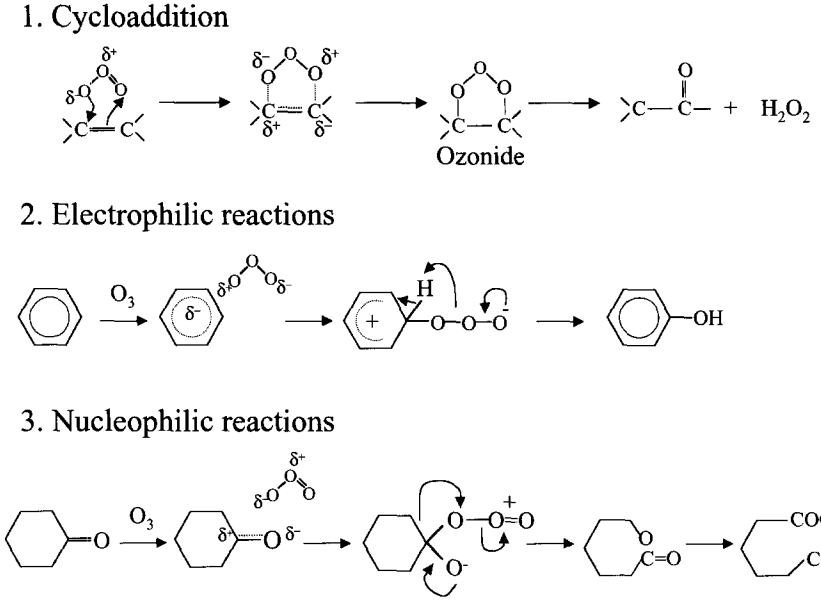

FIG. 5. Ozone in cycloaddition, electrophilic (Komissarov and Komissarova, 1973) and nucleophilic reaction (Leffler, 1949).

reported that the half-life of ozone was much longer in double-distilled water (> 85 min) at 20°C than in distilled or tap water (~ 20 min). Kim (1998) found that the half-life of ozone at 25°C in deionized and tap water was 12 and 6 min, respectively. The half-life of ozone in a solution containing 0.01 mol perchloric acid is ~ 18 h at 25°C and in the presence of 0.001 mol iron perchlorate is ~ 83 h. The half-life of free radicals is commonly measured in microseconds.

C. OZONE REACTIVITY

1. Molecular ozone

Ozone undergoes three types of reactions in organic solvent media (Bailey, 1978): (1) dipolar cyclo-addition with unsaturated carbon–carbon bonds; (2) electrophilic reaction with aromatic compounds, amines and sulfides having strong electronic density; and (3) nucleophilic reaction with carbons carrying electron-withdrawing groups (Figure 5). Therefore, the molecular ozone reactions are selective and limited to unsaturated aromatic and aliphatic compounds as well as to specific functional groups.

2. *Products of decomposition – free radical species*

Ozone gas is very unstable and decomposes quickly in the air. Because of its instability, the gas is generally produced at the point of application, sparged in water and applied immediately in a closed system. The following discussion addresses ozone decomposition in aqueous solutions. At low concentrations, auto-decomposition of dissolved ozone is an apparent first-order reaction with respect to ozone (Masschelein, 1982; Finch *et al.*, 1988; Peeters *et al.*, 1989). Auto-decomposition of ozone is accompanied by the production of numerous free radical species such as hydroperoxyl ($HO_2^{\cdot}$), hydroxyl ($^{\cdot}OH$) and superoxide ($^{\cdot}O_2^-$) radicals (Adler and Hill, 1950; Hoigné and Bader, 1975). The high reactivity of ozone is attributed to the oxidizing power of these free radicals. According to Jans and Hoigné (1998), when a mole of aqueous ozone decomposes, ~ 0.5 mol of $^{\cdot}OH$ is produced, regardless of whether the transformation is catalyzed by hydroxide anions (i.e. elevated pH), addition of H_2O_2, or exposure to UV

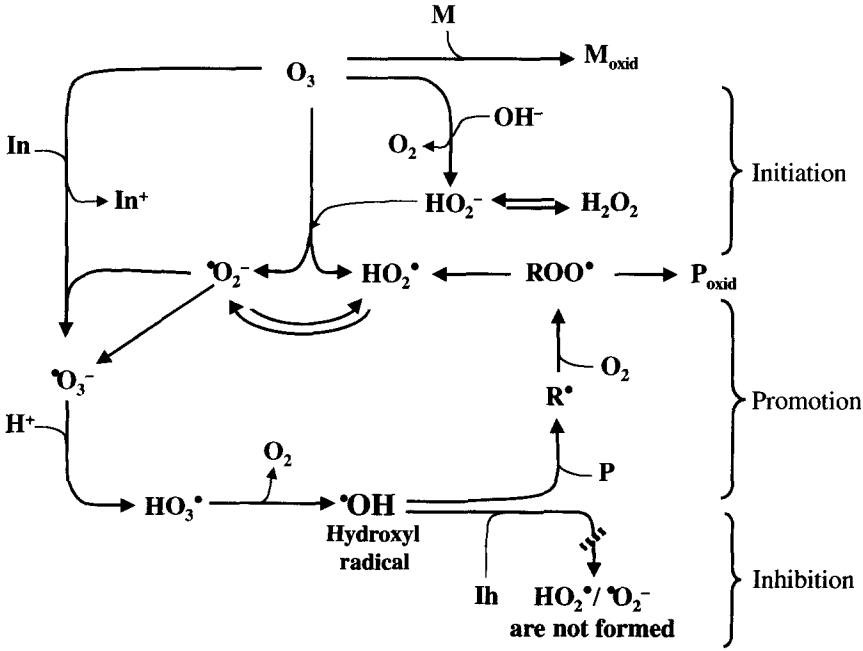

FIG. 6. Ozone decomposition reactions (adapted from Jans and Hoigné, 1998). M, Solute; In, initiator (e.g. OH^-, HO_2^-, Fe^{2+}, $HCOO^-$, –SH, UV); P promotor (e.g. O_3, –SH, R–CH_2OH, Aryl); Ih, inhibitor (e.g. Alkyl, HCO_3^-, CO_3^{2-}, t-BuOH, –SH).

irradiation. The hydroxyl radical is an important transient species and chain propagating radical. The reactions of hydroxyl radical with many substrates are very fast (Hoigné and Bader, 1975). Typical rate constants for reactions of hydroxyl radical with organic solutes are in the range 10^8 to 10^{10} M^{-1} s^{-1} (Farhataziz and Ross, 1977). Ozone decomposition occurs in a chain reaction process (Figure 6) including initiation, propagation, and termination steps (Weiss, 1935; Staehelin *et al.*, 1984; Jans and Hoigné, 1998). The initiators are compounds capable of inducing the formation of the superoxide radical ($^{\cdot}O_2^-$); these include hydroxyl ions (OH^-), hydroperoxide ions (HO_2^-), and some cations and organic compounds (e.g. glyoxylic acid, formic acid and humic substances). Ultraviolet radiation at 253.7 nm also can initiate the free-radical generation process. Promotion reactions regenerate the superoxide radical from the hydroxyl radical. Common promoters include aryl groups, formic acid, glyoxylic acid, primary alcohols and humic acids. Phosphate species are important inorganic promoters. The superoxide anion also can promote the decomposition of ozone. The inhibition reactions lead to the consumption of OH radicals without regenerating $^{\cdot}O_2^-$. Some of the common inhibitors include bicarbonate and carbonate ions, alkyl groups, tertiary alcohols and humic substances (Hoigné and Bader, 1985). Antioxidants such as tocopherol and ascorbic acid from food also can scavenge the free radicals and thus block the chain reaction.

3. Reactions with inorganic compounds

Minerals, metal ions, hydroxyl ions and halogens (e.g. chlorine) catalyze ozone decomposition and this increases ozone demand (Alder and Hill, 1950; Hewes and Davidson, 1973; Hoigné and Bader, 1976). Reaction of ozone with inorganic compounds found in water usually follows a first-order kinetics, with regard to ozone and the oxidizable compound. Ozone oxidizes ferrous (Fe^{2+}) into ferric (Fe^{3+}) species, which precipitate in water as ferric hydroxide, $Fe(OH)_3$, and is easily removed by filtration. Similarly, the manganous ion (Mn^{2+}) is oxidized by ozone into the manganic (Mn^{4+}) state, forming manganic oxide (MnO_2), which also precipitates out and can be filtered. These reactions are important for the removal of contaminant metals from drinking water (Dore, 1989). The oxidation–reduction potential values for ozone, chloride, bromide and iodide are 2.07, 1.49, 1.33 and 0.99 V, respectively (Table I). Hence, ozone can oxidize chloride ions slowly, but it reacts moderately with bromide and rapidly with iodide ions, producing elemental bromine and iodine, respectively (Haag and Hoigné, 1983).

III. MEDIUM FOR OZONE TREATMENT

When applied in food processing, ozone gas is used for food storage applications and the aqueous form is used in the surface decontamination of food equipment or packaging materials. Municipal water is commonly used for various washing purposes and dissolution of sanitizers; therefore, this water is the medium of choice for most aqueous ozone applications in food processing. The properties of the treatment medium considerably affect the efficacy of ozone treatment of food. Ozone demand, for example, resulting from dissolved substances in municipal water should be met before the desired sanitizing action occurs. The properties of the medium of significance to ozonation efficacy (i.e. temperature, relative humidity, residual ozone and ozone demand) will be addressed in this section.

A. TEMPERATURE

Several researchers have tested the relationship between ozone efficacy and treatment temperature, but their results seem inconclusive. Kuprianoff (1953) found that ozone was more effective against microorganisms when applied at low (< 10°C) than at high temperatures. Herbold *et al.* (1989) also reported that the effectiveness of ozone against hepatitis A virus (HAV) and *E. coli* diminished when the temperature increased from 10°C to 20°C. Katzenelson *et al.* (1974), however, indicated that lowering the temperature from 5°C to 1°C had only a minor effect on the inactivation kinetics of microorganisms. According to Kinman (1975), there is no difference in the disinfection rate by ozone when applied at 0°C or 30°C. Achen and Yousef (2001) treated apples inoculated with *E. coli* O157:H7 in bubbling ozone water at 4, 22 and 45°C for 3 min. The researchers found that residual ozone concentration following the treatments were 36, 22 and 18 mg L^{-1}, respectively, and no significant difference in ozone efficacy between the treatments at three different temperatures was found ($P > 0.05$). The disagreement among researchers may be due to the changes in ozone properties at different temperatures. A decrease in the temperature of an aqueous medium increases the solubility and stability of ozone. On the contrary, an increase in temperature enhances the reactivity of residual ozone. The relative contribution of these two factors (solubility/stability and reactivity) to ozone efficacy may vary with the experimental setup.

B. RELATIVE HUMIDITY

High humidity is needed for inactivation of microorganisms by ozone gas. It is believed that hydration of dry microorganisms in humid atmospheres

makes them susceptible to ozone. The optimum relative humidity (RH) for microbial inactivation by gaseous ozone is 90–95%, and the gas loses its bactericidal effect at $\leq 50\%$ RH (Kuprianoff, 1953). Ozone, however, decomposes more rapidly at high than at low RH values. Elford and van den Ende (1942) used low ozone concentrations and long exposure time at variable relative humidity to disinfect airborne microorganisms. At $< 45\%$ RH, the germicidal power of ozone was negligible. Inactivation was substantial at high humidities even when ozone concentration was < 0.1 mg L^{-1}. Ewell (1946) also demonstrated that microorganisms were killed more readily by ozone in an atmosphere having high rather than low RH. Hoffman (1971) 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. Ozone, therefore, is an effective sanitizer only against well-hydrated microbial cells. J. G. Kim and A. E. Yousef (Table II, unpublished data) found a similar reaction of ozone in a powdered, food-grade, anticaking agent containing natural contaminants. Application of 200 ppm gaseous ozone caused a minimal decrease in the microbial load of the anticaking agent with a water activity (a_w) of ≤ 0.84. When an anticaking agent that contained a_w of 0.96 was treated with 150 ppm gaseous ozone, the microbial load decreased by more than 2 log units. Application of 300 ppm gaseous ozone decreased the microbial load of this agent to an undetectable level. When a_w of a drier anticaking agent

TABLE II

INACTIVATION BY GASEOUS OZONE OF NATURAL MICROBIAL CONTAMINANTS ON SILICA-BASED ANTICAKING AGENTS WITH DIFFERENT WATER ACTIVITIES AND PH VALUES

Anticaking agent	a_w	pH	Ozone dose (ppm)[a]	Count (CFU g^{-1})
1	0.84	8.03	0	2.7×10^2
			200	1.7×10^2
1 (water added)	0.95	8.23	0	8.2×10^3
			150	3.0×10^1
			300	< 10
2	0.96	3.16	0	5.0×10^3
			150	1.5×10^1
			300	< 10
3	0.10	7.00	0	3.4×10^3
			200	2.3×10^3

CFU, colony-forming unit.

[a]μg gaseous ozone per g powder.

was increased from 0.84 to 0.95, ozone was as effective in decreasing the microbial load as it was in the product that naturally contained a high water activity. Microbial contaminants in the powder were mostly fungal and bacterial spores, which can survive in a suboptimal growth environment.

C. RESIDUAL OZONE

The term "residual ozone" is used in this chapter to refer to the detectable concentration, in the treatment medium, of ozone after it has been applied to the targeted food product. The effectiveness of ozone against microorganisms depends on the amount applied, but more so on the residual ozone in the medium. The stability of ozone under application conditions and the presence of ozone-demanding material in the treatment medium greatly affect the level of residual ozone available for disinfection of the food product. Venosa (1972) 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 sanitization. Therefore, in addition to the applied dose, the availability and the decay of ozone during the course of the treatments should be reported, otherwise the actual effective dose used may be overestimated.

The results of the following studies illustrate the importance of monitoring residual ozone. Izat *et al.* (1990) chilled eviscerated broiler carcasses in chlorinated water (20 ppm) or ozonated water at 1.7°C to 4.4°C. The oxidation-reduction potential (ORP) of the control (chlorinated water) was 900 mV and this value decreased as carcasses were introduced into the water. The ozone generator produced 20 g h^{-1}, resulting in an average ORP of 270 mV in the chiller water. After the first 20 carcasses were chilled, total microbial and presumptive coliform counts were significantly higher (unexpectedly) in the ozonated side of the chiller than in the control side. This suggests that either insufficient ozone was generated or the ozone was not remaining in solution for a sufficient time to affect bacterial numbers. In another study, shrimp meat was inoculated with *Vibrio cholerae*, *V. parahaemolyticus*, *Flavobacterium aquatile*, *Pseudomonas aeruginosa*, *P. putida*, *P. fluorescens*, *E. coli*, *Salmonella typhimurium* and *Staphylococcus aureus*. Inoculated meat was immersed in ozonated, 2% saline solution (2.9–4.8 mg L^{-1} ozone, 5°C) and flushed with ozone at 150 mL min^{-1} continuously for 60 min (Chen *et al.*, 1992). Ozone concentration decreased by more than 1.4 mg L^{-1} within 15 min but gradually increased thereafter. Reduction of bacterial count during the first 15 min of flushing was < 1 log unit except for *E. coli*, which had a 2-log unit decrease. A low ozone

concentration, therefore, is ineffective for disinfection of food products when extraneous organic matter is present. Bullock *et al.* (1997) used ozone to treat water in a recirculating rainbow trout (*Oncorhynchus mykiss*) culture to reduce the heterotrophic bacterial counts in system water and to prevent bacterial gill disease (BGD). Applied ozone was 25 or 36–39 g per kg of feed. Less than 90% reduction of *F. branchiophilum* in water or on gill tissue was achieved but BGD outbreaks were prevented. The limited decrease in bacterial count may be attributed to the short exposure time to ozone (35 s contact chamber) and rapid loss of oxidation caused by suspended organic matter in the medium.

D. OZONE DEMAND OF THE MEDIUM

When compared with other treatment media, pure water has the least ozone demand. Impurities in water react with applied ozone and generate demand. Some of these impurities may initiate ozone decomposition (Hoigné and Bader, 1976); these include glyoxylic acid, formic acid and humic substance. Kim (1998) bubbled several types of water used in the laboratory with ozone gas (1.1 mM) at a flow rate ~ 30 mL min^{-1} (Figure 2). Ozone dissolved faster in deionized and distilled water than in tap water, resulting in a higher maximum ozone concentration in the former water. The solubility of ozone was > 2-fold greater in deionized and distilled water than in tap water. Yang and Chen (1979) reported that the bactericidal efficacy of ozone was lower in Ringer solution, and in solutions containing NaCl (5%) or egg albumin, compared with distilled water. The tested substances decomposed ozone and thus decreased the amounts of residual ozone available for reaction with microorganisms. According to Restaino *et al.* (1995), death rates of some microorganisms in ozonated water, which contained organic matter, were not significantly affected by 20 ppm soluble starch but were significantly reduced by the addition of 20 ppm bovine serum albumin (BSA). Residual ozone in water containing BSA was significantly lower than in deionized water and water with soluble starch. Achen (2000) varied BSA concentration in *E. coli* O157:H7 suspension and treated the mixture with ozone. The researcher observed no inactivation of the pathogen when > 0.1% BSA was added (Figure 7). Ogawa *et al.* (1990), however, found that addition of 0.5 g loamy soil per liter of ozone solution (1.5–3.8 mg L^{-1}) did not affect the ability of ozone to inactivate spores of *Mucor piriformis*, *Botrytis cinerea* and *Phytophthora parasitica*. Antioxidants originating from food may generate ozone demand by scavenging radicals formed during ozone decomposition. Food additives such as acids, surfactants or sugars can stabilize or destabilize ozone, depending on their properties.

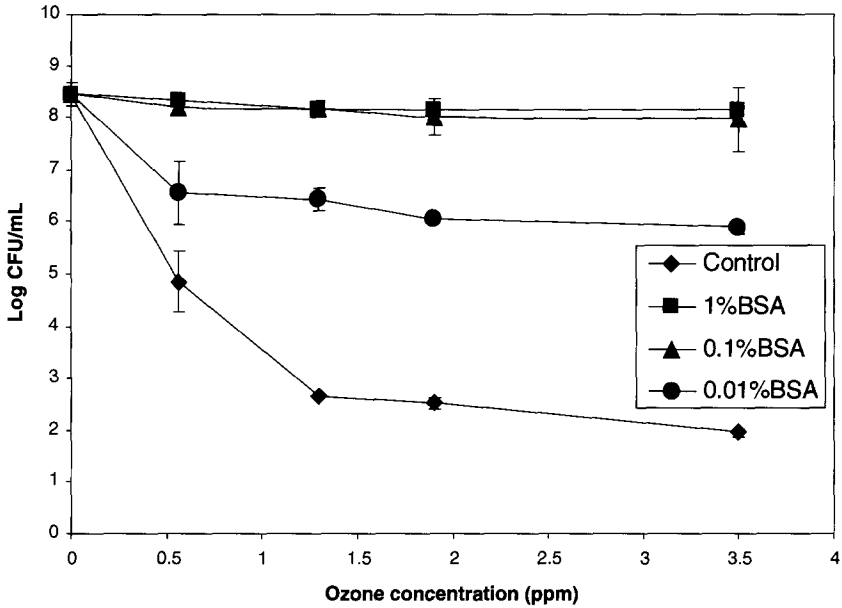

FIG. 7. Counts (CFU mL^{-1}) of *Escherichia coli* O157:H7 after exposure to different concentrations of ozone at 4°C for 20 s, in the presence of bovine serum albumin (Achen, 2000).

IV. REACTOR AND EQUIPMENT CONSIDERATIONS

For the treatment of food with aqueous ozone, the sanitizer must be transferred from the gas to the liquid phase. The design of treatment chambers and diffusion systems is important to maximize ozone transfer for the intended purpose and to make the process economically feasible. Dissolution/contacting units to provide aqueous ozone for application vary, depending on the specific functions of ozone at the points of application. A number of techniques are available for dissolution of ozone in the liquid. These include conventional fine bubble diffusion, turbine mixers, injectors, packed columns, spray chambers, deep U-tubes, porous plate diffuser contactors, and submerged static radial turbine contactors (Bellamy *et al.*, 1991).

The mass transfer of ozone occurs via diffusion through the gas–water interface. Favorable conditions for ozone mass transfer include high concentration of ozone in the carrier gas and high pressure (Gomella, 1972). According to Henry's law, high pressure above the process liquid

increases ozone solubility. White (1986), however, emphasized the importance of contactor efficiency, since maintaining high partial pressures of ozone above the process liquid is sometimes not attainable. Decreasing the diameter of the ozone bubbles increases the total area of exchange and the contact time between water and gaseous ozone (Harris, 1972). Meddows-Taylor (1947) defined "contact value" as the total area of gas bubbles multiplied by the time required to rise a unit distance. The author found that bubbles with a diameter of 0.1 cm have ~ 32 times more contact value than those with a diameter of 1.0 cm. In our experimental work (e.g. Kim and Yousef, 2000b), a sparger with 10 μm pore size was used effectively to decrease the bubble diameter of ozone gas and to increase the transfer rate of gaseous ozone into the water phase. In addition, stirring was carried out during ozonation to ensure sufficient turbulence.

Efficient ozone use in food processing can be achieved by adding a filtration apparatus prior to the contactor. Filtration may keep the level of nontarget demand substances to a minimum and improve ozone dissolution in the contactor (Hampson and Fiori, 1997). Prefiltration of poultry chiller water to decrease the organic load prior to ozone treatment is also recommended for optimum reduction of microbiological levels and efficient use of ozone (Sheldon, 1986; EPRI, 1999). When Sheldon (1986) filtered spent poultry processing water with diatomaceous earth (DE) prior to ozonation, the researcher obtained high-quality water. The filtration–ozonation combination decreased COD, total solids, fats/oil/grease (FOG), total aerobic microorganisms, coliforms and *Salmonella* spp. by 87%, 65%, 95%, 3 log units, 2.7 log units and 3 log units, respectively. A similar combined treatment was applied on whole-bird rinse water from commercial poultry processing plants (Sheldon and Chang, 1987). Measurable reductions in levels of COD (92%), absorbance at 280 nm (88%), total solids (59%), total volatile solids (82%), aerobic plate count (> 3 log units), coliforms (2 log unit), *E. coli* (2 log units), and *Salmonella* spp. (3 log units) were detected following the filtration–ozonation treatment. Filtration was more effective and rapid when DE rather than sand was used in removing contaminants from processing water.

A. OZONE INTERACTION WITH PROCESSING EQUIPMENT

Ozone not only reacts with contaminants (including microorganisms) in the treatment medium and food, it may also interact with the reactor and equipment materials. Efficacy of ozone treatment, therefore, may be influenced by the type of materials used to manufacture the equipment.

TABLE III
COMPATIBILITY OF DIFFERENT MATERIALS WITH OZONE[a].

Material	Theoretical rating[b]
304 stainless steel	Good
316 stainless steel	Excellent
Aluminum	Good
Bronze	Good
Copper	Excellent
ABS plastic	Good
Acetal (Delrin)	Fair
CPVC	Excellent
EPDM	Excellent
Hypalon	Excellent
Hytrel	Fair
Kel-F	Excellent
LDPE	Fair
Polycarbonate	Excellent
Polypropylene	Good
PTFE (Teflon)	Excellent
PVC	Good
PVDF (Kynar)	Excellent
Silicone	Excellent
Viton	Excellent
Natural rubber	Severe effect
Neoprene	Fair
Nylon	Severe effect
Buna N (Nitrile)	Severe effect

[a]Cole Parmer, Vernon Hills, IL.
[b]Ozone concentration not specified.

1. Reaction with metals

Ozone oxidizes metals except gold, platinum and iridium to oxides of the metals in their highest oxidation states. Ozone converts oxides to peroxides, sulfides to sulfates, carbon to carbon dioxide, and NH_3 to NH_4NO_3. In most oxidation reactions, ozone is reduced to molecular oxygen. Ozone at high concentrations corrodes equipment, but such high concentrations are found only inside the generator or in the ozone-to-water contacting system. Materials used in food processing are usually compatible with ozone at low concentrations (Table III). Stainless steel (e.g. 316L) is corroded less by ozone than by chlorine (Greene *et al.*, 1999; Singh and Singh, 1999). Glass-lined steel (Grosse and Streng, 1960) and steel with a phosphated inside surface (Waller and McTurk, 1965) are

resistant to ozone and suitable for maximum half-life storage of ozone. According to a recent report (Viera *et al.*, 2000), ozone in aqueous solution at 0.1 and 0.2 ppm, did not affect containers made of stainless steel and titanium, whereas copper alloys were susceptible to corrosion.

Metals commonly promote the decomposition of ozone and some of these reactions are catalytic. Good catalysts for ozone decomposition include iron, particularly if rusted, zinc, mercury, platinum and silver (Horvath *et al.*, 1985). Large specific surfaces of absorbents such as activated carbon, molecular sieves, silica gel and activated alumina, strongly catalyze the decomposition of ozone (Mahieux, 1962). Greene *et al.* (1999) reported that pulsing ozone into water at ambient temperature for 20 min per day for 7 days caused greater weight loss of aluminum, carbon steel, copper, 304 stainless steel and 316 stainless steel samples than that observed in the untreated controls; however, weight loss for carbon steel only was significant ($P < 0.05$). Severe pitting was noted on ozone-treated copper samples when observed by scanning electron microscope. Black striations were observed on ozone-treated carbon steel surfaces. Brass and copper also should be avoided for concentrations > 1.0 ppm aqueous ozone (Hampson, 2000). In an ozone atmosphere of 25–40 mg m^{-3}, metal surfaces must be protected by appropriate ozone-resistant painting; however, gas mixtures containing ozone ≤ 5 mg m^{-3} cause minimal corrosion (Kuprianoff, 1953). The author recommended stainless steel and anodized aluminum for construction of ducts and pipes that carry ozone.

2. *Reaction with rubber and plastics*

Common plastics used in food processing are generally resistant to ozone (Table II); these include polychlorotrifluoroethylene (EFTFE), polydichlorodifluoroethylene (PDFE), polytetrafluoroethylene (PTFE), polyvinylidenefluoride (PVDF), polyvinylchloride (PVC), and silicon tubing and gaskets (Grosse and Streng, 1960; Mahieux, 1962). Some plastic materials (e.g. PVC and polyethylene, PE) are useful in applications where low ozone concentrations are used. Rubber in seals, pipes and other components reacts actively with ozone, leading to a total disintegration into powder. Synthetic rubbers, however, are resistant to ozone (Horvath *et al.*, 1985). Fluorinated hydrocarbon lubricants have a good resistance to ozone, but polymers of monochlorotrifluoroethylene are the most suitable as lubricants. Silicon grease is adequate for short-term use, but it is oxidized on extended exposure to ozone. The sealing materials on doors and windows of fruit storage rooms should be made of ozone-resistant synthetic materials (Kuprianoff, 1953).

V. APPLICATION OF OZONE IN FOOD PROCESSING

Although ozone is highly effective against microorganisms in pure cell suspensions, it is unlikely to be used directly in food containing high ozone-demand materials. Organic constituents of such food compete with microorganisms for ozone, and thus high doses of this agent may be needed for effective elimination of microorganisms. These high levels of ozone may also alter sensory attributes, and adversely affect the acceptability of food. Current ozone applications in the food industry are mostly related to decontamination of processing water. Ozone, however, has been used with mixed success to inactivate microbial contaminants on meat products, eggs and dry food. Ozone is most suitable for treatment of solid food with intact surfaces such as fresh vegetables and fruits.

A. TARGETED MICROORGANISMS

Gaseous and aqueous ozone, at a low dose and with short contact time, is effective against numerous bacteria, molds, yeasts, parasites and viruses (Kim *et al.*, 1999b; Rodgers *et al.*, 1999). The efficacy of ozone as a sanitizer, however, depends on the target microorganism and treatment conditions. Microorganisms inherently vary in sensitivity to ozone. Ozone was tested recently against Gram-positive vegetative cells, bacterial spores, mold conidiospores and yeast ascospores that are commonly isolated from fruits and known to spoil fruit juices. Results show that bacterial spores are the most resistant and bacterial vegetative cells are the most sensitive to ozone (Kim and Yousef, 2000a). Spores of *Bacillus* spp. varied in susceptibility to ozone (Khadre and Yousef, 2001b). Among eight *Bacillus* spp. tested, spores of *B. stearotherophilus* were most resistant, while spores of *B. cereus* were most sensitive to ozone.

The physiological status of the treated microorganism may affect its susceptibility to ozone. Stationary-phase cells are more resistant to ozone than are cells from the exponential phase. The resistance of microorganism to ozone is greater with natural microflora on food than with microorganisms frequently cultured in the laboratory (Kim *et al.*, 1999a). E. T. Ryser (personal communication) compared the efficacy of sanitizers against natural and artificial contaminants on produce. In produce inoculation studies, ozone (3 ppm) treatments for 5 min decreased the microbial population ≥ 5 log units. In contrast, naturally occurring background populations generally decreased ~ 3 log units after similar treatments. Dormant microbial cells from a dry environment are extremely resistant to gaseous ozone (J. G. Kim and A. E. Yousef, unpublished data). Washing microbial cells

decreases their resistance to ozone because of the removal of ozone-demand material from the cell surface (Schechter, 1973).

Bacteria in biofilms have a polysaccharide architecture that protects them from antiseptics (e.g. hydrogen peroxide and chlorine-releasing preparations) and antibiotics (Dixon, 1998). Microorganisms attached to inert surfaces are less susceptible to the effect of chemical sanitizers than their free-living (planktonic) counterparts (Le Chevaillier *et al.*, 1988). Lethal dose increases when cells have complex envelopes and capsules, specially when cells are in a clump/biofilm. A higher lethal dose is required for spores than for vegetative cells. Existing colonies and clumped cells on the surface of food are hard to destroy because the outer layer of the clump protects inner cells. Mechanical treatment may help break the biofilm structure and increase accessibility of ozone to inner cells (Khadre and Yousef, 2001a).

B. INACTIVATION KINETICS AND MECHANISMS

Ozone kills microbial cells rapidly so that inactivation rates are difficult to measure. Difficulties in obtaining meaningful kinetic parameters have been addressed in a recent publication (Kim and Yousef, 2000b). Uniform procedures to measure inactivation kinetics and establish dose–response plots also were discussed. Suitable indicator microorganisms or spores should be used to measure ozone efficacy. Khadre and Yousef (2001b) suggested *Bacillus stearothermophilus* spore as an indicator of ozone sanitization.

A clearer understanding of the mechanism of inactivation is needed to optimize the effectiveness of ozone and supporting technologies. Ozone activity is likely related to its molecular form (Hunt and Marinas, 1997) or intermediate reactive species such as free radicals and singlet oxygen (Kanofsky and Sima, 1991). It appears that ozone causes damage to the following cellular constituents: (1) unsaturated lipids in the microbial cell envelope; (2) the lipopolysaccharides layer of Gram-negative bacteria; (3) intracellular enzymes; and (4) microbial genetic materials. Earlier studies suggest that ozone reacts with microbial cell membranes (Giese and Christenser, 1954). Ozone is believed to cause the oxidation of lipids on the cell envelope of bacteria (Murray *et al.*, 1965; Scott, 1975). Further oxidation leads to leakage of intracellular cell contents, damage of genetic material and death (Prat *et al.*, 1968; Shechter, 1973). Ozone reacts with cell dehydrogenases (Ingram and Haines, 1949), DNA (Scott, 1975) and RNA (Kim *et al.*, 1980). Khadre and Yousef (2001b) examined spores of *B. subtilis* with the electron microscope after these spores were treated with ozone. The authors observed damage to the outer spore coat layer, which may serve as a primary target of ozone.

Electron microscopic analysis revealed damage to cellular structures after ozone treatment (Kim, 1998; Dave, 1999; Khadre and Yousef, 2001b). Damage was more pronounced in Gram-negatives, *P. fluorescens* and *E. coli* O157:H7, than in Gram-positives, *Leuconostoc mesenteroides* and *Listeria monocytogenes* (Kim, 1998). When treated with ozone under similar conditions, Gram-positive bacteria seemed to lose only some mucoid material outside the cell wall, whereas Gram-negative cells tended to collapse and lose cellular components. Therefore, ozone at low concentrations damages the outer membrane of Gram-negative bacteria and thus causes dramatic changes in cell structure. Similar concentrations of ozone cause less visible damage to the cell wall of Gram-positive bacteria, but the agent causes intracellular damage and effectively inactivates these cells (Kim, 1998).

Kim (1998) also tested the injury of microorganisms by ozone. The degree of injury varied depending on the microorganism, ozone concentration and exposure time. Maximum injury occurred at ozone concentrations that caused mild lethality. Combined treatment of ozone and pulsed electric field (PEF) resulted in a synergistic lethal effect against *E. coli* O157:H7 and *L. monocytogenes* (Unal *et al.*, 2001). It was suggested that the synergy may result from cell injury during the ozone treatment and rapid inactivation of injured cells when they were subsequently treated with PEF.

C. OZONE AS AN ALTERNATIVE SANITIZER IN FOOD PROCESSING

The food industry has traditionally used chlorine to limit microbial growth on processing equipment and in wash water. Fresh-cut produce is commonly treated with chlorine to extend product shelf-life and minimize the risk of foodborne pathogens. Despite the benefits of chlorine, chlorination may lead to the formation of toxic or carcinogenic chlorinated organic compounds in water (Brungs, 1973; Page *et al.*, 1976; Kirk and Mitchell, 1980) and food, or on food contact surfaces (Wei *et al.*, 1985). Although considerable chlorine-related research has been done to determine optimal parameters for reducing pathogens and extending product shelf-life, its effectiveness on fruits and vegetables remains variable and unpredictable (Nguyen-the and Carlin, 1994). Some studies suggest that chlorine, at high concentrations, causes only modest inactivation of pathogens on food. When Brussels sprouts were treated with 200 mg L^{-1} chlorine, the count of *L. monocytogenes* decreased by only 2 log units (Brackett, 1987). Beuchat and Brackett (1990) also reported that treating shredded lettuce with chlorine did not prevent the growth of *L. monocytogenes* after the lettuce was packaged in modified atmosphere. Chlorine dioxide treatments only inactivated 1.1 log units of *L. monocytogenes* population on lettuce and 0.4 log unit on cabbage (Zhang and Farber, 1996). To improve the safety

of sprouts, treatment of seeds with 20 000 ppm chlorine has been recommended (US-FDA, 1999).

Given the drawbacks of chlorination, researchers are actively seeking alternatives to chlorine use in food processing. Chlorine dioxide offers several advantages over chlorine as a sanitizing agent and disinfectant (Dychdala, 1991). Based on availability of oxidative species in solution, the oxidation capacity of chlorine dioxide is 2.5 times greater than that of chlorine. Compared with chlorine, chlorine dioxide is also slower in dissociation/hydrolysis, stable over a broader pH range, less corrosive to metal equipment, and less likely to form chlorinated by-products and potential carcinogens. Chlorine dioxide has been used for many years as a water disinfectant because of its bactericidal qualities and its ability to react with humic substances without the formation of trihalomethanes.

Hydrogen peroxide and peracetic acid are potential alternatives to chlorine as a food sanitizer. In a study of various sanitizers for beef brisket adipose tissue, Gorman *et al.* (1995) found that hydrogen peroxide is one of the most effective sanitizers. While Bundegaard-Nielsen and Nielsen (1996) reported that peracetic acid was not effective as a fungicide, Orth and Mrozek (1989) demonstrated the effectiveness of peracetic acid in reducing numbers of several foodborne bacteria.

Ozone is emerging as a viable alternative to chlorine. The strong germicidal action and the high oxidation potential are some of ozone's properties that make it an attractive alternative to the traditionally used chemical disinfectants. In addition, ozone decomposes rapidly to oxygen and it leaves no toxic residues; this makes ozone a favorable sanitizer for users concerned about the environment. Ozone, compared with chlorine, is a more powerful and efficient antimicrobial agent against spores, fecal and pathogenic microorganisms, and viruses in an environment containing a high proportion of organic matter (Gomella, 1972). Ozone is also effective for pesticide residue reduction (Ong *et al.*, 1996), food preservation, shelf-life extension, equipment sterilization and improvement of food plant effluents (Horvath *et al.*, 1985; Hampson, 2000). The superiority of ozone over chlorine compounds was reported by many researchers (Kessel *et al.*, 1943; Scarpino *et al.*, 1972; Korich *et al.*, 1990). Of particular importance to the fruit and vegetable industry is a report indicating that greater than 90% of *C. parvum* oocysts added to water were inactivated after 5 min of exposure to 1 ppm ozone (Korich *et al.*, 1990). In contrast, approximately 90 min of exposure to 80 ppm chlorine were required to achieve similar results. Ozone is effective in surface decontamination of fresh produce. Ozone was recommended recently as an alternative to chlorine (Kim, 1998) and hydrogen peroxide (Khadre and Yousef, 2001b).

D. FOOD PROPERTIES AND OZONE APPLICABILITY

1. Food composition

Commodities with different chemical composition require different ozone dose for effective sanitization. Fresh meat, which contains high fat contents, for example, requires more ozone than do fruits and vegetables, which contain low fat and high carbohydrates. Fournaud and Lauret (1972) treated beef with gaseous ozone during refrigeration and thawing to reduce the surface microorganisms. Gaseous ozone concentrations as high as 500 ppm caused little microbial inactivation. The authors attributed treatment inefficacy to the reaction of ozone with fat and proteins in the meat rather than with the contaminating microorganisms. Kaess and Weidemann (1968b) found that the ozone consumption per unit area of fatty surface tissue was considerably smaller than that of muscle tissue.

2. Surface structure

The nature of the surface of food contributes substantially to the efficacy of ozone treatment. Bacteria on poultry carcasses are located primarily on skin surfaces, within feather follicles and on exposed muscle surfaces. Microorganisms located within the feather follicles are generally protected from the bactericidal action of disinfectants, as shown by relatively small reduction in carcass microbial counts during washing and ozonation (Barnes and Impey, 1968). Bullock *et al.* (1997) used an ozone treatment in a recirculating rainbow trout (*Oncorhynchus mykiss*) culture system and prevented bacterial gill disease (BGD) outbreaks but found that the causative bacterium, *F. branchiophilum*, was still colonizing gill tissues. In recent studies, bubbling ozone in wash water for 3 min was effective in reducing microbial counts on the surface of apples by up to 3.7 log units (Klingman and Christy, 2000; Achen and Yousef, 2001). Ozone treatment, however, was less effective in decontaminating the calyx and stem areas of the apple. Spraying water on these areas prior to the ozonation helped dislodge the cells and reduced the counts by 1.5 log CFU g^{-1}. When *E. coli* O157:H7 was permitted to attach to the apple surface, the efficacy of disinfection by ozone diminished.

In conclusion, strongly attached surface microorganisms and those attached to areas that are not freely exposed to ozone cannot be eliminated by mere dipping in ozonated water. In addition, microorganisms embedded in product surfaces are more resistant to ozone than those suspended in water. Therefore, when ozone is applied in food processing, good contact between the sanitizer and the target microorganisms on the treated food

should be ensured. A variety of methods have been used to accomplish this goal including stirring, pumping, fluming, bubbling, sonication, abrasion and pressure washing.

3. Release of exudates

Most fruits and vegetables have a hard protective layer of peel, skin or rind, and the outer surface is usually covered with a waxy material. These products commonly have a limited ozone demand. However, in minimal processing, fresh vegetables and fruits are usually trimmed, peeled (or cut if necessary) and washed, thus tissues are exposed and cellular fluids are released that increase ozone demand. When hot water or steam is used for blanching, vitamins, flavors, colors, carbohydrates and other water-soluble components are inevitably leached (Ihl *et al.*, 1998); this released organic matter constitutes ozone-demand. Treating meat with ozone is a high ozone demand process, since the meat surface has crevices and it leaches ozone-demanding organic substances such as fats and proteins.

4. Injuries and wounds

Gaseous ozone treatment was not effective in decreasing infection in inoculated wounds in apples (Schomer and McColloch, 1948). Ogawa *et al.* (1990) readily inactivated spores of *B. cinerea* on the surface of non-injured tomato fruit using 3.8 mg L^{-1} aqueous ozone for 10 min. However, spores placed on the surface of injured tomato fruit were not inactivated. Smock and Watson (1941) reported that when spores were protected by moist surfaces of apple flesh or by other organic protectants, ozone had no effect on their germination. When pear fruits were wound-inoculated with *Penicillium expansum* and then treated with 5.5 mg L^{-1} aqueous ozone for 5 min, levels of decay were similar to those of a control treated with water only (Spotts and Cervantes, 1992). This suggests that plant tissues and extracellular biochemicals at wound sites react with ozone and render it ineffective.

E. OZONE APPLICATION AT DIFFERENT STAGES OF PROCESSING

Ozone may be applied directly on raw agricultural commodities at the pre-processing stage, during processing, or on the finished product while at storage. It is usually advantageous to apply ozone on the raw than the processed product. Whole grains, for example, require less ozone to disinfect than does the powder product (Naitoh *et al.*, 1987). Raw solid food commonly has intact surface and most of the natural contaminants are limited

to the surface. Elimination of readily accessible surface contaminants is feasible using most sanitizers including ozone. Sanitizers, however, may differ in dealing with contaminants that are attached or embedded in food surfaces. Aqueous ozone can be used to decontaminate beef and beef brisket fat (Gorman *et al.*, 1995), poultry meat (Dave, 1999), salmon (Goche and Cox, 1999), apples (McLoughlin, 2000; Achen and Yousef, 2001), strawberries (Lyons-Magnus, 1999), lettuce (Kim *et al.*, 1999a), broccoli and cauliflower (broccoflower) (Hampson and Fiori, 1997) and other commodities. Microbial studies typically show a reduction of 2 log total count and a significant reduction of spoilage and potentially pathogenic species that are most commonly associated with fresh fruits and vegetables. When these raw foods carry high organic loads, effectiveness of ozone treatment is likely to diminish. Multiple-stage wash system may be needed in this case with a pre-wash and a rinse preceding the ozone treatment.

Some researchers used ozone to treat ingredients before they are included into food formulation. Kim *et al.* (1993) treated various spices, used to prepare Kimchi, with ozone and improved the fermentation of the final product. J. G. Kim and A. E. Yousef (unpublished) also used ozone to decontaminate the ingredients of fruit juices such as high-fructose corn syrup. These researchers speculated that ozone treatment of ingredients, rather than the final juice, reduces ozone usage and minimizes damage to the sensory quality of the final product. Naitoh *et al.* (1989) reported that the treatment of wheat flour with ozone inhibited microbial growth in namamen products and increased their storage life.

Application of ozone in the processing facility to minimize environmental contaminants has been studied (Naitoh, 1993; Hampson, 2000). Combinations of ozone with other oxidants such as hydrogen peroxide were used to sanitize packaging films (Gardner and Sharma, 1998), a confectionary plant (Naitoh, 1989), and hatchery equipment (Whistler and Sheldon, 1989). Ozone decreased surface flora by ~ 3 log units when tested in wineries for barrel cleaning, tank sanitation, and clean-in-place (CIP) operations (Hampson, 2000). Some wineries have embraced the use of ozone for multiple purposes, including barrel and tank cleaning and sanitation, CIP systems, and for general purpose sanitation (Duca, 1999; Hampson, 2000). Aqueous ozone, at 1.5 ppm, was tested in a food-processing facility. The treatment decreased the microbial load on a stainless steel kettle, table top, shroud, plastic shipping container, floor surface and drain (Hampson, 2000). The author reported reductions of up to 3 log total plate count on floor surfaces and shrouds, but less than 1 log unit in floor drains. The author recommended using ozone in a complementary sanitizing regime to maintain the overall cleanliness and sanitation of wineries and any other food-processing facility.

Gaseous ozone can be used to extend the shelf-life of food during storage. Ozone minimized growth of surface contaminants on meat (Greer and Jones, 1989), grapes (Sarig *et al.*, 1996) and broccoli florets (Zhuang *et al.*, 1996) when the gas was applied during storage of these commodities.

VI. SELECTED FOOD APPLICATIONS

A. RAW POULTRY AND MEATS

Count and diversity of the microbial population dictates the shelf-life of raw poultry and meats. In addition to numerous spoilage microorganisms, these products occasionally carry pathogenic microorganisms such as *Campylobacter* spp., *Salmonella* spp., *L. monocytogenes* and pathogenic *E. coli*. The types and number of microorganisms present on raw poultry and meats depend on the microorganisms that colonize the gastrointestinal tract. Food contamination with these microorganisms can occur at multiple steps along the food chain including slaughtering, handling, storage and distribution (Zhao *et al.*, 2001). Use of sanitizers on carcasses and cut meat is currently limited. Chlorine may be used in the poultry chiller tanks or as spray on carcasses, but alternative and effective sanitizers have been pursued.

Several investigators tested decontamination of beef and beef brisket fat by ozone; results were variable (Gorman *et al.*, 1995, 1997; Reagan *et al.*, 1996). Other research groups found that gaseous ozone minimized or prevented growth of microorganisms on the meat surface (Kaess and Weidemann, 1968a; Greer and Jones, 1989; Mitsuda *et al.*, 1990). Numerous investigators have demonstrated the microbicidal efficacy and safety of ozone for use in washing poultry carcasses (Barnes and Impey, 1968; Yang and Chen, 1979; Sheldon and Brown, 1986; Caracciolo, 1990; Izat *et al.*, 1990; Jindal *et al.*, 1995), reconditioning poultry chiller water (Sheldon, 1986; Sheldon and Chang, 1987; Waldroup *et al.*, 1993; Diaz and Law, 1999), and sanitizing hatchery equipments (Whistler and Sheldon, 1989). Advanced oxidation processes, including ozone and adjuncts such as hydrogen peroxide and UV radiation, enhanced the efficacy of ozone as an antimicrobial control agent in poultry chiller water (Diaz and Law, 1999; EPRI, 1999). Prefiltration of the chiller water prior to ozone treatment is recommended for optimum reduction of microbiological levels and efficient use of ozone (Sheldon, 1986; EPRI, 1999).

The presence of *Salmonella enterica* ser. Enteritidis in shell eggs has serious public health implications. Several thermal and chemical treatments have been developed to control or eliminate this pathogen in

eggs. Yousef and Rodriguez-Romo (2001) used ozone at low temperatures and under mild pressure for cold sanitization of shell-eggs. Shell-eggs were externally contaminated with *S.* Enteritidis so that shells contained ~ 10^6 CFU g^{-1}. Eggs were treated with gaseous or liquid ozone for 1–20 min, at 4–25°C and 0–15 psi (0–100 kPa). Gaseous ozone treatment without pressure decreased the count of *S.* Enteritidis on shells by 2.2–2.7 log units. Treating contaminated eggs with gaseous ozone for 10 min at 22–25°C and 15 psi decreased *Salmonella* population by more than 5 log units. Such a treatment may be used industrially to produce "cold-sanitized" eggs. Cox *et al.* (1995) patented a "hyperpasteurization" process, which uses vacuum, heat and ozone, to eliminate *Salmonella* spp. from the contents of shell eggs. This method includes, heating shell eggs at higher than 54.4°C for longer than 15 min with subsequent application of ozone. According to this report, the combined treatment extended the shelf-life and reduced the microbial load of shell eggs.

B. FRUITS AND VEGETABLES

There are many steps in the food production chain with multiple potential sources of contamination at each step. For example, dirty irrigation water, manure fertilizer, and improper worker hygiene have been cited as probable causes for pre-harvest contamination of fresh fruits and vegetables. Following harvest, improper handling and storage, use of contaminated wash water, processing equipment and transportation facilities as well as cross-contamination from other produce contribute to the microbial hazards associated with fresh fruits and vegetables. Of special concern is the quality of wash water and the potential hazard associated with cross contamination (Tauxe *et al.*, 1997). Following cutting, shredding and slicing of fresh-cut fruits and vegetables, the loss of surface integrity can lead to penetration and rapid growth of microorganisms. In a study on processing conditions of chopped lettuce and coleslaw, shredders were identified as the major source of in-plant contamination (Garg *et al.*, 1990). Other contributing factors include the expansion of production facilities and longer food marketing chains that allow the distribution of more heavily contaminated produce to wider populations (Tauxe *et al.*, 1997). Refrigerated fresh-cut produce is susceptible to microbial spoilage (Nguyen-the and Carlin, 1994) and growth of pathogenic microorganisms (Beuchat, 1995). Ozone application for improving the safety and extending the shelf-life of fresh-cut produce seems feasible. Improving water reusability by the fruit and vegetable processing industry is an additional benefit of ozone application. Ozone-sanitized produce has been introduced recently in the United States market after several years of developing and testing (TVA,

2001). The following are examples of studies and successful attempts to sanitize fresh produce with ozone.

1. Apples

Apples are subject to contamination with pathogenic microorganism on the farm. Use of contaminated apples to produce unpasteurized apple juice and cider resulted in *E. coli* O157:H7 foodborne infections (Besser *et al.*, 1993; CDC, 1996b). The Food and Drug Administration (FDA) now regulates the production of cider with recommendations for the pasteurization of apple cider and other juice products or the use of alternative processing steps to reduce the counts of the pathogen in question by 5 log units (FDA, 1998). Use of effective sanitizers on whole apples prior to pressing is a feasible option. Chlorine and hydrogen peroxide with surfactants and isothiocyanate have been investigated as sanitizers (Beuchat *et al.*, 1998; Lin *et al.*, 2000). In a recent study, Achen and Yousef (2001) inoculated apples with *E. coli* O157:H7 prior to treatment with an aqueous solution containing 21–28 mg L^{-1} ozone. Decontamination treatments were more effective when ozone was bubbled during apple washing than by dipping apples in pre-ozonated water. Maximum decreases in surface counts of *E. coli* O157:H7 were 2.6–3.7 log units, compared to unwashed inoculated controls. However, counts of *E. coli* O157:H7 decreased by less than 1 log unit in the stem-calyx region by the ozone treatment. Rinsing the apples with an inorganic wetting agent (tetrasodium pyrophosphate) increased the efficacy of the ozonation process. The wetting agent may have enhanced the contact between ozone and bacterial cells that are attached to the hydrophobic surface of the apple, decreased cell attachment on the stem and calyx areas, and assisted in exposing entrapped cells to ozone. The authors speculated that in conventional apple washing environments, the efficacy of ozone against microbial contaminants may become limited because of the high organic loads in the washing tanks resulting from debris, soils, and fruit saps; these contaminants impose an ozone demand.

2. Lettuce

Kim *et al.* (1999a) tested ozone against natural contaminants in fresh lettuce and results were compared with those obtained from chlorine treatment. Ozone (1.3 mM) or chlorine (1 mM) inactivated mesophilic and psychrotrophic bacteria by 1.4 and 1.8 log units in 3 min, respectively. Counts of these microorganisms on lettuce, from a different batch, decreased 3.9 and 4.6 log units, respectively, during 5 min of ozone treatment. In another

experiment, shredded lettuce was treated with gaseous ozone, or mixed with aqueous solution of ozone (1 : 20 w/w) with or without bubbles. Results show that bubbling gaseous ozone in water, combined with stirring, is the most effective ozonation method for shredded lettuce.

3. Alfalfa sprouts

Alfalfa sprouts received great attention in recent years owing to the incidence of pathogens in this product and associated disease outbreaks. In 1998, the FDA issued a statement warning consumers of high-risk groups to avoid consumption of sprouts due to the potential health hazard associated with these products. J. M. Boff and A. E. Yousef (unpublished data) determined the effectiveness of ozone in reducing the natural flora on alfalfa sprouts. Treatment of alfalfa sprouts with ozone for 5 min decreased their microbial load by 1.2 log units. An additional 0.8 log unit decrease in population was observed when ozonation was accompanied with agitation. As an alternative ozonation process, alfalfa seeds were pretreated with ozone and the microbial count during seed germination and growth was monitored. Additionally, ozonated water was used to water the sprouts twice daily during the growth period. The initial microbial count on the seeds was ~ 10^5 CFU g^{-1} and ozone treatment decreased the count by ~ 2 log units. The difference in count between ozone-treated and nontreated seeds diminished during the growth period and the population in both treatments reached ~ 10^9 CFU g^{-1} after 4 days of seed incubation. Most of the growth of the natural flora occurred in the first 2 days after setting. Treatment with ozone water during the growth period only temporarily decreased the count, but the counts after 4 days of growth were identical in ozone-treated and nontreated sprouts.

An end treatment consisting of bubbling ozone into the sprouts for 2.5 min decreased the microbial count from 5.0×10^9 to 2.0×10^7 CFU g^{-1}. Alternatively, the sprouts were placed in ozonated water (30–32 ppm) and stirred for 20 min; the average count decreased to 4.8×10^7 CFU g^{-1}. The latter method may be preferable to the former one because of better quality and texture of the resulting sprouts. In conclusion, ozone treatments, as tested in this study, are not sufficient to bring about a substantial reduction in the microbial population on sprouts.

4. Fruit juice ingredients

Technological advances in citrus processing led to development of convenient, shelf-stable, ready-to-drink juices. Survival of heat-resistant bacteria and fungi during pasteurization of juice can be a serious concern

to citrus processors. Ozone is potentially useful in inactivating heat-resistant spores in juice components with low ozone demand. Therefore, the efficacy of ozone against spores of *Alicyclobacillus acidocaldarius*, *Neosartorya fischeri* and *Zygosaccharomyces bailii*, which are known to be problematic in juices, was tested in selected juice ingredients (Kim and Yousef, 2001). Fruit juice components, high fructose corn syrup (HFCS), orange juice concentrate (OJC), and pine apple juice concentrate (PJC), spiked with cells or spores (*c.* 10^7 mL^{-1}), were treated with gaseous ozone. The sensitivity of spores to ozone varied depending on the type of spores and the juice component. The ozone dose required for inactivating 5 log units of *A. acidocaldarius* spores was 0.31, 0.28 and 0.41 mg ozone per mL spore suspension in HFCS, OJC and PJC, respectively. The ozone dose for inactivating 5 log units of *N. fischeri* spores was 0.12–0.51 mg mL^{-1}, depending on the juice ingredient. The amount of ozone required to inactivate *N. fischeri* spores was four times greater for PJC than for HFCS. In order to achieve a decrease of 5 log units of *Z. bailii* spores, 0.04–0.24 mg mL^{-1} was needed, depending on the juice component. Inactivation of *Z. bailii* spores by ozone was faster in HFCS than in other juice components even though HFCS has higher solids than the other components. In conclusion, the ozone dose required to achieve a 5-log unit decrease of the targeted microorganisms varied from 0.04 to 0.5 mg mL^{-1} with *A. acidocaldarius*, and *N. fischeri* spores were three to four times more resistant to ozone than were *Z. bailii* spores. The authors do not recommend applying ozone directly to juice concentrates or reconstituted juice. Ozone, however, may be safely applied to the HFCS. Ozonation can also be combined with different inactivation technologies to minimize the changes in product quality while maximizing the inactivation of contaminants.

C. FISH PROCESSING AND STORAGE

Ozone was tested for decontaminating shrimp (DeWitt *et al.*, 1984), mussels (Abad *et al.*, 1997), and various fish such as jack mackerel (Haraguchi *et al.*, 1969), sockeye salmon (Lee and Kramer, 1984), Japanese flounder (Mimura *et al.*, 1998) and rockfish (Kötters *et al.*, 1997). The antimicrobial efficacy of ozone was equal to or better than that of chlorine in some applications studied (Arimoto *et al.*, 1996; Goche and Cox, 1999). Ozone reduced disease incidence in hatcheries with less mortality and shorter growth cycles (Blogoslawski *et al.*, 1993; Arimoto *et al.*, 1996; Bullock *et al.*, 1997). The absence of adverse sensory effects and harmful oxidation by-products confirms the desirability of ozone use in processing fish products for human consumption. Ozone treatment of shrimp meat extract, however, was ineffective against microorganisms in the product (Chen *et*

al., 1992). Ozone may have reacted with ozone-demand substances in the extract, instead of microbial cells.

D. DRY FOOD AND FOOD INGREDIENTS

Gaseous ozone can eliminate *Bacillus* spp. and *Micrococcus* spp., which are dominant in cereal grains, peas, beans and spices, by up to 3 log units, depending on concentration, temperature and relative humidity (Naitoh *et al.*, 1988). The surface area of the dry food is an important factor for the effectiveness of ozone treatment. Cereal flour and ground pepper, for example, require higher concentration of ozone and longer contact time than whole cereal and pepper to achieve the same degree of microbial inactivation (Naitoh *et al.*, 1989; Zagon *et al.*, 1992). In addition to antimicrobial effects, ozone destroys or greatly reduces aflatoxins from peanut (Dollear *et al.*, 1968) and cottonseed meal (Rayner *et al.*, 1971) and oxidizes odors produced during dehydration of onions and garlic (McGowan *et al.*, 1979). Ozone treatment may cause lipid oxidation (Naitoh *et al.*, 1988), decrease amino acid (e.g. thiamine) content (Naitoh *et al.*, 1989) and essential oil content (Zhao and Cranston, 1995), and contribute negative effects on the sensory quality of some dry food.

J. G. Kim and A. E. Yousef (Table II, unpublished data) used ozone to inactivate natural contaminants in a silica-based anticaking agent. High water activity with limited organic matter provided suitable conditions for growth of bacteria and fungi in this product. Ozone gas at 30–40 ppm was sufficient to sterilize an anticaking agent but failed to decontaminate other types of the substance. The authors hypothesized that water activity is an important factor for cell inactivation by ozone in the anticaking agent.

E. PACKAGING MATERIAL AND FOOD CONTACT SURFACES

Microbial contaminants on food packaging materials are commonly small in number, but they may survive conventional decontamination processes and cause food spoilage. Currently, sterility of packaging materials is achieved by several methods including heat, hydrogen peroxide and UV radiation (Stefanovic and Dickerson 1986; Yokoyama 1990; Gardner and Sharma 1998). Sterilization by hydrogen peroxide is a tedious and variable method (Yokoyama 1990), and unacceptable levels of hydrogen peroxide residues may remain and interact with some of the polymer in the packaging material (Stefanovic and Dickerson 1986; Castle *et al.* 1995). Sanitization of equipment and food-contact surfaces is essential for safety and quality of processed food. When biofilms develop on wet food contact surfaces such as those made from stainless steel, the micro-

organisms inside the biofilms are usually protected from sanitizers (Frank and Koffi, 1990; Carpentier and Cerf, 1993; Dixon, 1998). Hence, alternative methods for decontamination of packaging materials and stainless steel are being sought.

A multilaminated aseptic food packaging material and stainless steel were treated with ozone to inactivate natural contaminants, bacterial biofilms (*P. fluorescens*) and dried films of *B. subtilis* spores (Khadre and Yousef, 2001a). Sterility of the multilaminated packaging material, which contained a low natural, mostly mesophilic, contaminants was achieved when it was treated with 5.9 mg L^{-1} ozone in water for 1 min. Counts of bacteria in dried films decreased by 4.6–5.1 log units for the multilaminated packaging material and 5.5 log units for stainless steel when treated with 5 mg L^{-1} aqueous ozone for 1 min. Dried films of spores (10^8 per 6.3 cm^2 surface) decreased below detection (< 10 spores per 6.3 cm^2 surface) by application of 13 mg L^{-1} aqueous ozone for the multilaminated packaging material and 8 mg L^{-1} in the case of the stainless steel. Repeated exposure to ozone and agitation during the treatment were needed to decrease effectively the population in biofilms. Ozone inactivated *P. fluorescens* in biofilms more effectively on stainless steel than on the multilaminated packaging material. The relatively low efficiency of ozone against bacteria in the biofilm, compared to that in dried films, is probably due to the tenacious adherence of bacteria to the surface of packaging material when biofilms are formed. It is concluded that ozone is an effective sanitizer with potential applications in the decontamination of packaging materials and equipment food-contact surfaces. Removal of biofilms by ozone, however, requires additional mechanical action during the treatment.

F. PESTICIDES ON AGRICULTURAL COMMODITIES

Use of pesticides on fruits and vegetables is crucial for insuring high-quality products. Residues of these pesticides, however, raise the concern of consumers and effective means to remove these residues are sought. The use of chlorine, ozone, chlorine dioxide and peracetic acids as postharvest treatments has been effective in remediating several different pesticides on apples (Ong *et al.*, 1996; Siler, 1998; Hwang, 1999). These same treatments also have the added benefit of reducing microbial populations on the surface of fruits and vegetables by up to 5 log units (Rodgers *et al.*, 1999). Organic food processors and consumers of organic food also are concerned about the presence of chemical residues, including chlorine and chlorinated by-products. However, these residue levels are likely reduced by ozonation in solution.

VII. COMBINATION TREATMENTS

Molecular ozone, or free radicals resulting from its degradation, interact with pollutants or microorganisms and cause their destruction. Direct reaction of ozone with organic compounds is generally relatively slow. Therefore, most ozone reactivity is associated with the radical-chain reaction rather than with the direct reaction with solutes (Hoigné and Bader, 1979). Typical rate constants for reactions of the hydroxyl radical with organic compounds are in the range 10^8 to 10^{10} M^{-1} s^{-1} (Farhataziz and Ross, 1977). Thus, microorganisms are inactivated faster in the presence of radicals formed during ozone decomposition than by ozone molecules themselves because of the higher reaction rates of the former.

In order to improve ozone action, free radical generation and the combination of ozonation with other technologies have been studied. Advanced oxidation process techniques are designed to promote the formation of hydroxyl free radicals, resulting in increased microbicidal activity above that of ozone itself. Effective ozonation procedures are also being developed through improved delivery systems in order to overcome the physical barriers that diminish the efficacy of sanitization of products, and to maximize the biocidal action of ozone.

A. OZONE AND HYDROGEN PEROXIDE

The combination of ozone and hydrogen peroxide in aqueous solution generates hydroxyl free radicals (Figure 6). Combinations are obtained by adding the necessary amount of hydrogen peroxide to the water being treated and then passing the solution through an ozone-contacting apparatus (Graham, 2000). Hydrogen peroxide is a weak acid, which partially dissociates into hydroperoxide ion in aqueous solutions.

$$H_2O_2 + H_2O \rightarrow HO_2^- + H_3O^+$$

$$O_3 + HO_2^- \rightarrow {}^{\cdot}OH + {}^{\cdot}O_2^- + O_2$$

The hydrogen peroxide molecule reacts slowly with ozone, whereas the hydroperoxide anion is highly reactive (Taube and Bray, 1940). Consequently, the rate of ozone decomposition by hydrogen peroxide increases with increasing pH. The reaction rate of ozone decomposition with H_2O_2 is theoretically 100 000 times greater than that of ozone decomposition initiated by hydroxyl ions. Therefore, very low concentrations of H_2O_2 are kinetically effective in initiating O_3 decomposition.

For optimum oxidative performance, a specific weight ratio of peroxide to ozone is required to destroy each pollutant. For example, the taste- and odor-causing compounds, geosmin and 2-methylisoborneol in potable water, require a peroxide to ozone ratio of about 1 : 0.3. Pesticides sometimes require weight ratios as high as 1 : 0.8. Therefore, it is advisable to determine experimentally the optimum peroxide to ozone ratio that is most suitable for a given application (Graham, 2000). Glaze *et al.* (1987) found that the rate of oxidation of organic load in water decreases when the weight ratio of peroxide to ozone is greater than one. The Electric Power Research Institute (EPRI, 1999) evaluated the efficacy of ozone and hydrogen peroxide combinations in poultry chiller operation. When a broiler was rinsed with chiller overflow ultrafiltrate containing 1–2 mg L^{-1} ozone and 0.5 mg L^{-1} hydrogen peroxide, the average decrease in total count was more than 2 log units.

B. OZONE AND CHLORINE

Processors of fresh-cut produce maintain a minimum chlorine concentration in wash water to ensure the efficacy of the treatment. Therefore, chlorine residues are monitored as a critical control point in these processes. Processors who look for ozone as an alternative to chlorine treatments are concerned that the efficacy of sanitization cannot be similarly measured, since ozone treatments may not result in measurable residues.

The potential interaction of ozone and chlorine should be considered when combining these two sanitizers in a processing line. Ozone oxidizes residual chlorine in water to form chlorate and perchlorate, which are weaker oxidants (Kolle, 1968; Siddiqui, 1996). However, Buydens and Fransolet (1971) did not notice any interaction of ozone and chlorine. Therefore, sequential application of ozone and chlorine is potentially a useful combination treatment in such facilities.

C. OZONE AND OTHER GASES

Tahoe Food Technology, Inc. (1998) invented an apparatus that produces a continuous stream of mixed gas containing ozone, carbon dioxide and argon. Navel oranges were treated with this gas mixture for 2 h in a sealed chamber to control bean thrips, red scale and fuller rose beetle at 20°C, 30% RH and 9.5 psi (66 kPa). The concentrations of ozone, carbon dioxide and argon were 4.0, 10.0 and 1.0%, respectively. All adults, larvae and eggs (fuller rose beetle only) were killed in the process. The treated naval oranges were incubated for 28 days after treatment to ensure that all three life cycles had been destroyed.

Mitsuda *et al.* (1990) tested the decontamination of fresh cucumbers with a mixture of ozone and carbon dioxide gases in polyvinylchloride film bags. The concentration of ozone gas generated was 20–40 g m^{-3}. The food in each bag was exposed for 5 min to ozone, carbon dioxide, and their mixture. The best results were obtained when mixing ozone and carbon dioxide at 3 : 1 and 2 : 1 (v/v). After 14 days storage, ~ 1 log unit decrease of bacteria was obtained with 2 : 1 and 1 : 1 mixtures. The survival of microorganisms during storage was lower for the mixed gases of ozone and carbon dioxide than it was for the individual gases. The authors reasoned that this synergistic action was a result of the quenching effect of carbon dioxide on the chain decomposition reaction of ozone, and by the bacteriostatic effect of carbon dioxide.

D. OZONE AND HEAT

Ozone degrades rapidly with heat; therefore, simultaneous application of these two preservation factors is ineffective against food microflora. Sequential application of ozone and heat, however, may be beneficial. J. G. Kim and A. E. Yousef (unpublished data) applied sublethal concentrations of ozone to *B. subtilis* spores and then measured thermal inactivation of pre-treated spores. Results (Table IV) show that ozone treatment greatly sensitized bacterial spores to heat. The D-value was decreased considerably by the sublethal ozone pre-treatment. Khadre and Yousef (2001b) examined ozone-treated bacterial spores using the transmission electron microscope. The authors found that treatment of spores with aqueous ozone (10 mg L^{-1}) for 1 min caused substantial damage to the outer coat layer. Therefore, weakening of the outer coats by sublethal ozone concentrations may have sensitized *B. subtilis* spores to heat.

E. OZONE AND ULTRAVIOLET RADIATION

An advanced oxidation process, based on a combination of ozone and UV radiation, enhances the antimicrobial action of ozone (Diaz and Law,

TABLE IV

D-VALUES (MIN) OF *BACILLUS SUBTILIS* SPORES AFTER PRETREATMENT WITH 6 PPM OZONE SOLUTION FOR 1 MIN AND SUBSEQUENTY HEATING AT 85–95°C

	Temperature		
Treatment	85°C	90°C	95°C
Control	294	74.6	27.0
Ozone	26.3	9.3	4.0

1999; EPRI, 1999). Ozone has a maximum absorption for UV radiation at 253.7 nm. In a gaseous phase enriched with water vapor, photolysis of ozone involves the release of a molecule of oxygen and an atom of oxygen (McGrath and Norrish, 1960); the latter may react with water to produce hydroxyl radicals. Treatment of water by ozone and UV combination is achieved by placing a UV bulb in the ozone-contacting chamber (Graham, 2000). As water flows through the chamber, the UV bulb is turned on to initiate the decomposition of ozone molecules to hydroxyl radicals.

Kruithof and Kamp (1999) applied advanced oxidation processes in drinking waters spiked with pesticides or with clostridia spores. Ozone/peroxide and UV/peroxide combinations rapidly destroyed the pesticides tested; however, ozone treatment alone was not effective. Clostridia spores (2.2×10^4 spores mL^{-1}) were resistant to ozone/peroxide and ozone/UV combination treatments, but, spore count decreased to below detection level with the UV/peroxide treatment.

Diaz and Law (1999) evaluated UV-ozonation system for the treatment of unscreened overflow poultry chiller water samples and obtained a more than 60% decrease in total plate count, coliforms and *E. coli*, and more than 80% of the light transmission of fresh water. Synergy between ozone and UV radiation accounted for >0.8 log unit decrease in total plate count. A synergistic bactericidal effect also was reported between ozone and UV radiation when poultry overflow chiller water, inoculated with nalidixic acid-resistant *S. typhimurium*, was treated with the combination for 4–8 min.

F. OZONE AND PULSED ELECTRIC FIELD

Unal *et al.* (2001) explored the potential enhancement of inactivation of *Lactobacillus leichmannii*, *E. coli* O157:H7 and *L. monocytogenes* by use of a pulsed electric field (PEF) when they are pretreated with ozone. The authors found a synergistic bactericidal effect. The *E. coli* count decreased by 3.6 log units and the *L. monocytogenes* count by 3.9 log units when these bacteria were treated sequentially with 0.75 mg ozone mL^{-1} and 15 kV cm^{-1}. However, ozone at 0.75 mg L^{-1} inactivated *E. coli* and *L. monocytogenes* by 1.8 and 3.0 log units, and PEF at 15 kV cm^{-1} inactivated the microorganisms by 1.8 and 0.8 log units, respectively. The synergy was more apparent at mild than severe doses of ozone, and when the combination treatment was applied to *Lb. leichmannii* as opposed to *E. coli* or *L. monocytogenes*. Oshima *et al.* (1997) treated *E. coli* with combinations of PEF and ozone. They reported that simultaneous application of PEF and ozone synergistically inactivated *E. coli*. Their

data, however, show that ozone and PEF combinations had an additive rather than a synergistic effect.

VIII. ANALYTICAL METHODS

Ozone can be produced by electric discharge, photochemical, chemical, thermal, chemonuclear, and electrolytic methods (Horvath *et al.*, 1985). The corona discharge method is commonly used to produce large amounts of ozone but the UV-based methods generate smaller yield and concentrations of the gas. The corona discharge produces ozone when a high-voltage alternating current is applied across a discharge gap in the presence of oxygen or air (Kim *et al.*, 1999b).

There are physical, physicochemical, and chemical methods for the measurement of ozone. Most of the ozone analytical methods are modifications of chlorine residual methods, which are based on determining the total oxidation in solution. Physical methods measure direct absorption in the UV, visible or infrared region of the spectrum. Physicochemical methods are dependent upon reaction outputs such as heat or chemiluminescence. Chemical methods quantitate products released from the reaction between ozone and a chemical reagent such as potassium iodide.

Determination of residual ozone in aqueous solutions is quite difficult because of the rapid decomposition of ozone, volatility from solution, and reactivity with many organic and inorganic chemicals. The iodometric method has been widely used (Gordon and Grunwell, 1983). This method, however, measures ozone and other oxidizing species present in an ozone reaction solution. In addition, several factors, including pH, buffer composition, buffer concentration, iodide ion concentration, sampling techniques and reaction time, affect the accuracy of the iodometric method. Hence, measurement of residual ozone by the iodometric method is not recommended. The indigo method currently is widely used for determining residual ozone in water and waste water (APHA, 1998); it is relatively sensitive, precise and fast, with a detection limit of 0.005 mg L^{-1} ozone (Bader and Hoigné, 1981). Indigo has a relatively high molar absorptivity (~ 20 000 mol^{-1} cm^{-1}) and the dye absorbs light at 600–610 nm. Ozone reacts selectively with the carbon–carbon double bond of the sulfonated indigo molecule; therefore, ozone measurement by this method is not affected by the presence of hydrogen peroxide, organic peroxides, manganous ions and oxidized species in the aqueous medium. For gaseous ozone measurements, a UV spectrophotometric method, which is based on UV absorbance at 258 nm and a molar absorptivity of 2900 mol^{-1} cm^{-1}, is most suitable.

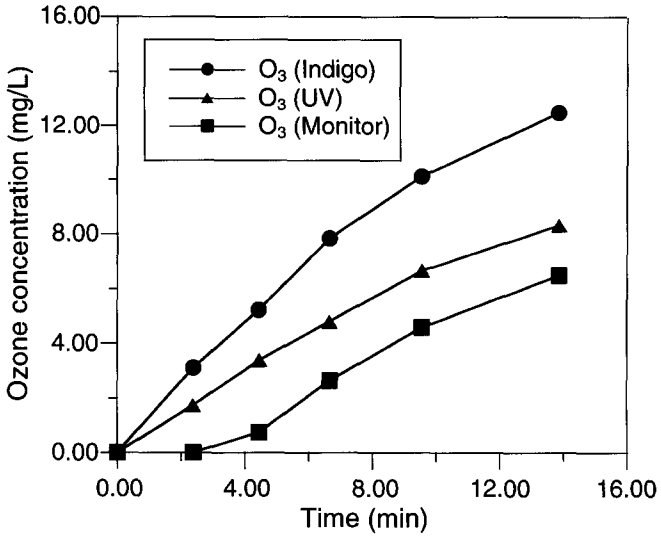

FIG. 8. Monitoring ozonation of water for 14 min using the indigo-based chemical method, a UV-spectrophotometric method, and a commercial ozone analyzer (Monitor).

In-line measurements of ozone are based on spectrophotometric, calorimetric or potentiometric methods. Several manufacturers produce instruments to monitor ozone concentration based on UV absorption. Calorimetric methods of ozone measurement depend upon decomposition of ozone in the presence of a catalyst. Residual ozone in water can also be measured by amperometric or potentiometric methods. The latter methods depend on the oxidation–reduction potential of ozone. In our laboratory, we compared ozone measurements using a commercial ozone analyzer (Ebara Jitsugyo Co., Ltd., Tokyo, Japan), the indigo-based chemical method (Hoigné and Bader, 1986) and a UV spectrophotometric continuous procedure (Kim and Yousef, 2000b). Results (Figure 8) show that ozone measurements by the commercial analyzer were consistently smaller, compared with those from the other methods (unpublished data). The length of the tube between the ozone reservoir and the analyzer seems to affect the discrepancy between the results. In addition, the length of the tube between the ozone reservoir and the UV spectrophotometer also cause variations in ozone concentration readings by this instrument. Since ozone is such a dynamic oxidizing agent, there should be a minimal time lag between collection of a sample and analysis. Consideration should also be given to the ozone demand of materials in contact with the sanitizer, e.g., the holding and handling apparatus.

IX. REGULATORY STATUS

Ozone use is permitted in many European and Asian countries. Ozone has been safely and effectively used in water treatment for several decades in European countries (Bryant *et al.*, 1992). Industrial use of ozone in the United States was limited to the removal of metal ions, color, taste and odors in water (O'Donovan, 1965). In 1975, the US-FDA permitted the use of gaseous ozone up to 0.1 ppm in meat-aging coolers and in 1982, ozone was approved as a Generally Recognized As Safe (GRAS) substance for treatment of bottled water (USDA, 1982). According to the 1982 ruling, other uses of ozone (e.g. in food) require a food additive petition. Subsequently, the United States Department of Agriculture (USDA) permitted the use of ozone in poultry chiller water (USDA, 1984). The Electric Power Research Institute and Agriculture and Food Technology Alliance submitted a direct food additive petition to FDA so that ozone could be used in food processing without limitations (Graham, 2000). In response to the petition, the FDA amended the food additive regulations to provide for the safe use of ozone in gaseous and aqueous phases as an antimicrobial agent on food, including meat and poultry (Federal Register, 2001). This approval should boost a broad use of ozone in food processing.

X. LIMITATIONS, TOXICITY AND SAFETY

The reactivity of ozone and the potential deterioration in the quality of treated product may limit uses of this sanitizer in food processing. Sensory attributes may be altered, depending on the chemical composition of food, ozone dose and treatment conditions. Surface oxidation of food by ozone results in discoloration, undesirable odors and oxidative spoilage. Ozone decreased vitamins and amino acid contents and increased lipid oxidation and activity of some enzymes such as superoxide dismutase, ascorbate peroxidase, and glutathione reductase in lettuce leaves (Kang *et al.*, 1999).

The acute and chronic effects of excessive exposure to ozone were investigated (Stockinger, 1965). Small concentrations of ozone gas in air (0.3 ppm) may cause discomfort to susceptible people. Scott and Lesher (1963) reported that as little as 0.02–0.04 mg L^{-1} can be detected by humans and 0.1 mg L^{-1} is objectionable to all normal humans because of irritation to the nose, throat and eyes. The respiratory tract is the primary site of ozone toxicity, where the gas may cause pulmonary congestion. Symptoms resulting from exposure to ozone include headaches and dryness of the throat, nose and eyes (Stockinger, 1965; Mustafa et al., 1980). Some researchers demonstrated that repeated exposures have progressively

lesser effects, suggesting that tolerance may develop (Nadel, 1979). Thorp (1950) 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. Menzel (1984) postulated that ozone may generate toxic substance when tissue proteins, unsaturated fatty acids or other components of food are oxidized. However, immersion of shrimp meat in saline containing 5 mg L^{-1} ozone, for 120 min, did not generate mutagens in the product (Chen *et al.*, 1992).

In a recent direct food additive petition, Graham (2000) argued that ozone can be used comparatively safely in industrial applications. According to the author, ozone is detected by human olfactory senses at concentrations as low as 0.01 ppm, and higher concentrations of the agent exert only temporary acute symptoms in humans. Since ozone has a high oxidation potential, disinfection can be accomplished with less concentration and shorter exposure time, compared to other oxidizing agents. Ozone is manufactured on-site at relatively low concentrations and pressures, therefore, an uncontrolled, widespread, and immediate release of large quantities of ozone is not possible. Graham (2000) also indicated that the relatively short half-life of ozone minimizes the persistence of the gas in the environment, and ozone breakdown product (diatomic oxygen) cause no harm.

When ozone is generated and used in food applications, precautions and personal safety always should be observed. Dissolving ozone in water is commonly accompanied by excess undissolved gas that remains entrapped in the solution (Hampson, 2000). Excess ozone should be degassed or separated from the water stream prior to delivery to equipment or the processing environment. Ozone detection and destruction systems and respirators are needed for the safety of workers in food processing facilities. Good manufacturing practice (GMP) and hazard analysis and critical control point (HACCP) systems are needed to control high ozone demand materials in food processing; this helps optimize ozone use in the processing facility. Workplace monitoring for ozone off-gas should be performed, and records should be maintained to ensure compliance with regulation.

Pryor and Rice (2000) discussed ozone exposure threshold limits. In the United States, current permissible exposure level-time weighted average (PEL-TWA) for ozone exposure in the work place environment is 0.1 ppm, as recommended by the American Conference of Governmental Industrial Hygienists (ACGIH, 1986) and adopted by the United States Occupational Safety and Health Administration (OSHA). Susceptible individuals can be exposed continually to this ozone concentration during a normal 8-h day/40-h working-week without adverse effects (CFR, 1997). The short-

term exposure limit is 0.3 ppm for an exposure less than 15 min and four times per day.

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