ABSTRACT

Interest in ozone has expanded in recent years in response to consumer demands for ‘greener’ food additives, regulatory approval and the increasing acceptance that ozone is an environmentally friendly technology. Ozone, a powerful oxidant, is effective against various kinds of microorganisms on fruits, vegetables, meat grains and their products. The multifunctionality of ozone makes it a promising food processing agent. Excess ozone auto decomposes rapidly to produce oxygen and thus leaves no residues in foods from its decomposition. Ozone as an oxidant is used in water treatment, sanitising, washing and disinfection of equipment, odour removal, and fruit, vegetable, meat and seafood processing. Ozone treatment assures the retention of sensory, nutritional and physicochemical characteristics of food. Treatment conditions should be specifically determined for all kinds of products for effective and safe use of ozone.

Keywords Ozone, greener, additives, oxidant, physicochemical.

Minimising pathogenic and spoilage microorganisms in fruits, vegetables and their products are a primary food-safety concern. Traditionally thermal processing methods are used to inhibit the pathogens. This technology, however, affects the quality of foods. Non-thermal processing prevents the food quality losses such as loss of original flavour, taste, appearance, colour, nutritional quality etc. In general, current sanitization technologies are crucial to maintaining the quality and enhancing the safety of fresh agricultural commodities but it is required to minimise the drawbacks and potentially hazards caused by the treatments to consumers. Promising results have been revealed in solving the problems of the food industry like microbes, pests, mycotoxin and pesticide residues by ozone application. Spontaneous decomposition without forming hazardous residues in the treatment medium makes ozone safe in food applications. If improperly used, ozone can cause some deleterious effects on products, such as losses in sensory quality.

Ozone is a strong oxidant and potent disinfecting agent. Disinfecting agents have widespread applications to assure safety and quality in the food industry. However, some of these agents, such as chlorine, are inefficient against some organisms, particularly at high pH or against spore-forming microbes. Furthermore, chlorine can react to form trihalomethanes, which are of concern for both human dietary safety and as environmental pollutants. Therefore, the food industry is in search of applications that are:

- Effective in inactivation of common and emerging pathogens, and removing toxic contaminants.
- Leading to less loss in product quality and ensure ‘freshness’.
- Adaptable to food processes and economically feasible.
- Environmental friendly

The bactericidal effects of ozone have been documented on a wide variety of organisms, including Gram positive and Gram negative bacteria as well as spores and vegetative cells. There are numerous application areas of ozone in the industry such as food surface hygiene, sanitation of food plant equipment, reuse of waste water, treatment and lowering biological oxygen demand (BOD) and chemical oxygen demand (COD) of food plant waste.

Properties and Characteristics of Ozone

Ozone was discovered and named by Schoenbein in 1840, but its applications for food treatment did not develop until much later. Ozone (O₃) is tri-atomic oxygen formed by addition of a free radical of oxygen to molecular oxygen. The three atoms of oxygen in the ozone molecule are
arranged at an obtuse angle, whereby a central oxygen atom is attached to two equidistant oxygen atoms; the included angle is approximately 116° and the bond length is 1.278 Å. The boiling point of ozone is 111.9 ± 0.3 °C, the melting point is 192.5 ± 0.4 °C, the critical temperature is 12.1 °C and the critical pressure is 54.6 atm (Manley and Niegoswki, 1967). Ozone exists in the gaseous state at room and refrigeration temperature and it is partially soluble in water. At room temperature, ozone is an unstable gas. Ozone readily degrades (Manley and Niegoswki, 1967) but has a longer half-life in the gaseous state than in aqueous solution (Rice, 1986). Ozone is relatively stable in air but highly unstable in water, decomposes in a very short time. It cannot be stored and must be generated continuously. The only result of ozone, when it decomposes, is oxygen; so, food products treated with ozone are free of disinfectant residue. It is readily detectable at 0.01–0.05 ppm level (Miller et al., 1978). It has a pungent, characteristic odour described as similar to “fresh air after a thunderstorm.” Ozone is a blue gas at ordinary temperature when generated from dried air, but colourless when generated from high-purity oxygen.

Purity of water usually affects ozone stability. Although ozone in pure water degrades rather quickly to oxygen, it degrades even more rapidly in impure solutions. Hill and Rice (1982) reported that approximately 50% of ozone is destroyed in 20 minutes at 20°C in distilled or tap water, whereas only 10% of ozone breaks down in 85 minutes in 20 °C double-distilled water. Ozone solubility in water is 13 times that of oxygen at 0–30 °C and it is progressively more soluble in colder water (Rice, 1986). Ozone decomposition is faster in higher water temperatures. Ozone is a toxic gas; toxicity is dependent on concentration and length of exposure (Pascual et al., 2007). At short-term exposure rates of 0.1–1.0 ppm, symptoms include headaches, nosebleeds, eye irritation, dry throat and respiratory irritation. At higher exposure levels (1–100 ppm), symptoms become more severe and include asthma-like symptoms, tiredness and loss of appetite.

**Generation of Ozone**

Ozone (O₃) is formed by a high energy input that splits the oxygen (O₂) molecule in the air into free radical oxygen. Single oxygen (O) molecules rapidly combine with available O₂ to form ozone (3 O₂ → 2 O₃ + heat and light). In nature, the source of this high energy is the ultraviolet irradiation from the sun and also lightning discharge. Since ozone is unstable, it splits back into oxygen molecule. Ozone can be generated on-site as required by several techniques, three of which are available commercially at the present time – corona discharge, UV radiation and electrolysis.

**Corona discharge or plasma technique**

The most commercially significant technique is by corona discharge (the so-called “silent electrical discharge” procedure). This is tantamount to producing synthetic lightning. In a corona discharge ozone generator, the feed gas (dried air, oxygen, or mixtures thereof), passes between two closely spaced electrodes (one of which is coated with a dielectric material) under a nominal applied potential of ~10 kV. A silent or barrier discharge occurs when the gas becomes partially ionized, resulting in a characteristic violet glow when air is the feed gas (with high purity oxygen the violet coloration is seldom observed).

There are two electrodes in corona discharge, the high tension and low tension (ground) electrodes, separated by a dielectric medium in a narrow discharge gap. When electrons have sufficient energy to dissociate the oxygen molecule, a certain fraction of these collisions occur and a molecule of ozone can be formed from each oxygen atom. A schematic diagram of ozone generation by corona discharge method is given in Fig. 3.1.

Efficiency of ozone production by corona discharge depends on the strength of micro-discharges which are influenced by a number of factors such as the gap width, gas pressure, properties of the dielectric and metal electrodes, power supply, and the presence of moisture. In weak discharges, a significant fraction of the energy is consumed by ions, whereas in stronger discharges, almost all of the discharge energy is transferred to electrons responsible for the formation of ozone. The optimum is a compromise that avoids energy losses to ions but at the same time obtains a reasonable conversion efficiency of oxygen atoms to ozone.

If air is used as the feed gas, it must be scrupulously dried and be free of traces of oils and greases (oxidized by ozone). Moist air gives rise to nitric acid in the ozone generator which will form nitric acid which will corrode the generator, requiring frequent maintenance and down time. If air is passed through the generator as a feed gas,
1–3 % ozone is made; using high-purity oxygen may yield as high as 16 % ozone (Rice et al., 1981).

**Physico-chemical or ultraviolet radiation**

The mechanism of photochemical production of ozone is similar to that which occurs in the stratosphere, that is, oxygen atoms formed by the photo-dissociation of oxygen by short wavelength UV radiation react with oxygen molecules to form ozone. Although the theoretical quantum yield of ozone by this technique is 2 %, in practice the actual yield is more on the order of 0.5 %, because the low pressure mercury lamps produce not only the 185 nm radiation responsible for the production of ozone, but also the 254 nm radiations that destroy ozone. Medium pressure UV that produces higher levels of 185 nm radiation produces more ozone. An advantage of generating ozone by UV radiation is that ambient air can be used efficiently as the feed gas. On the other side, quantities of ozone generated per 40-W UV bulb are low (0.5 g/h) at maximum concentrations of 0.25 wt %. However, these maximum ozone yields and concentrations cannot be attained simultaneously by the UV method. The low concentrations of ozone available from UV generators limit their applicability for water treatment to special applications. However, their use to generate ozone for air treatment can be effective.

**Electrolysis**

High current density electrolysis of aqueous phosphate solutions at room temperature produces ozone and oxygen in the anodic gas. Electrolysis of 68 wt-% sulfuric acid can produce 18-25 wt-% ozone in oxygen when a well-cooled cell is used. Although electrolysis of water can produce high concentrations of ozone, the output is low, and the cost is several times more than that of the corona discharge process. However, small electrolytic units are being used commercially for treatment of ultra-high purity waters in pharmaceutical and electronic industries.

**Components of Ozone System**

An ozonation system takes up air or feed gas from the atmosphere and concentrates oxygen for ozone generation. Ozone is generated, its concentration is analysed and passed to the treatment chamber (collector) for microbial inactivation. The excess ozone from treatment chamber is released into air as oxygen to prevent chance of health hazards. A complete ozonation system (gaseous ozone) for use in food processing plants consists of the following subunits:

**Oxygen concentrator**

For food processing plants, oxygen-enriched air (>90% O₂) is provided simply and conveniently by means of oxygen concentrators. These devices take in ambient air, automatically filter (remove dust particles), then separate and remove nitrogen (thereby leaving air considerably enriched with oxygen), which is also dried to below the desired maximum dew point (°4°C), all at the same time in one small device. These oxygen concentrators operate on the principle of pressure swing adsorption (PSA) drying. An air compressor pressurises the airflow and sends it through a molecular sieve (microscopic porous bead) bed that adsorbs or traps nitrogen and moisture, while providing oxygen-enriched air to the supply output of the concentrator. As the molecular sieve bed becomes loaded with nitrogen and moisture, they...
desorb to waste in vapour form to the environment, recovering the adsorption capacity of the sieve bed. Today the majority of ozone generating systems use oxygen as the feed gas because the concentrations of ozone produced are increased two to three times for the same energy expenditure. Most ozone equipment manufacturers have optimised their equipment for food processing plants to operate on oxygen feed gas.

**Ozone generator**

Ozone generators may be UV generators or Corona discharge generators. The quality of gas fed to an ozone generator can be a critical factor, particularly if ozone is produced by CD or plasma techniques. So CD generators require an air preparation prior to ozone generation. The ozone output is only slightly increased by increasing the oxygen content or drying the air. The two common types of gas preparation to feed CD ozone generators are oxygen and dry air. UV generators of ozone do not require any special air preparation.

If an air dryer is selected to feed a CD ozone system, make sure that the air preparation equipment is matched and sized to the ozone. Each ozone generator is designed to operate at an optimal flow rate depending upon size of ozone generator, and such information should be stated in the supplier’s equipment manual. With moisture present in the feed gas, the very corrosive nitric acid (HNO₃) is readily formed. Consequently, the gas feeding an ozone generator must be very dry (maximum “54 °C dew point), because the presence of moisture also affects ozone production, as well as leading to the formation of nitric acid. This very corrosive acid can destroy the internal parts of a CD ozone generator, which can cause premature system failure and will increase the frequency of required maintenance.

When using oxygen as the feed gas, the concentration of ozone produced is increased two to three times over ozone concentrations in dried air for the same energy expenditure. For example, dry air-fed CD generators of the size used in food processing plants produce 1–2 wt% ozone, but when fed high-purity oxygen, ozone concentrations of 3–6 wt% are produced for the same energy expenditure. Most ozone equipment manufacturers have optimised their equipment for oxygen feed gas. In some instances, ozone generators can produce as high as 20 wt% ozone (from oxygen), but the energy and other requirements are higher to produce ozone at this concentration. Properly designed and operated CD-ozone generators commercially available today are capable of producing kg/h quantities of ozone in gas phase concentrations of 1-5% by weight in air and up to 14% by weight in high purity oxygen. The advantage of a higher weight per cent ozone product is economics.

The process of electrically rupturing oxygen atoms to produce oxygen ions or atoms that combine with more oxygen to produce ozone liberates considerable heat. This heat generated during the process must be removed from the generator to avoid the reverse reaction (ozone reverting to oxygen) from taking over and decreasing the efficiency of ozone generation. Consequently cooling of the corona discharge ozone generator becomes a critical component in generator design. Normally cooling is provided by water, but there are air cooled CD-ozone generators commercially available, especially on smaller scale, appropriate for many food applications.

**Flow meters**

Mass flow meter provides accurate measurement of total oxygen gas flow from the oxygen concentrator to ozone generator. Ozone flow meter use small gas stream (less than 2 LPM) to measure ozone concentration and for regulation of gas flow through ozone analyser.

**Treatment chamber**

It is the collector where ozone enters from the top and returns though the bottom outlet after completing the exposure time. The chamber is air tight and avoids leakages. The dimension and type will be based on the mode of application of ozone and type of product being processed. The outlet is connected to ozone analyser and then to ozone destructor.

**Ozone analyser**

This device will be used for measuring the ozone concentration in per cent by weight, or g/m³ that enters and leaves the treatment chamber.

**Ozone destructor**

At the outlet of the destructor, excess ozone is destroyed, and the cleaned and decontaminated air is re-circulated to its intended enclosure or discharged to the ambient atmosphere. Excess ozone can be broken down into oxygen and send to the atmosphere in order to prevent any harm for
the worker. Ozone at high concentration is corrosive and toxic even at lower ppm. In this device, the excess ozone is allowed to release into the air such that the contact of both is controlled. Since ozone is unstable in air, decomposition of ozone takes place and oxygen is given out through the outlet. Destructor is an important part that assures non-hazardous by-product or waste in ozone treatment.

Data and analysis

The data such as exposure time, ozone concentration can be acquired using ozone analyser and used for analysis of results.

Modes of Application

There are many modes of application of ozone to the food product. The mode of application may be selected based on the type of food and the need of ozone treatment. Ozone generators are available based on the type of feed gas available using for ozone generation. The concentration and intensity varies with different modes used for the same effect. D.M Graham (2000) explains the mode of application of ozone as follows.

Application in aqueous phase

Ozone forms true solution with water. The solubility of ozone in water is based on Henry’s law, which states that ‘pressure applied to a vapour in equilibrium with a liquid is inversely proportional to the temperature’. Half-life of ozone in aqueous solution is less than that of gaseous ozone; so decomposes rapidly. The decomposition of ozone is very rapid in the presence of impure water. Ozone is only partially soluble in water; efficient transfer of ozone into solution requires the dispersion of gaseous ozone into small bubbles. This is accomplished in various types of positive pressure ozone contractors such as bubble diffuser/bubble columns, mechanically agitated vessels, turbine mixers, tubular reactors, in-line static mixers as well as negative pressure reactors (venturi) and injectors. In positive pressure devices, ozone gas under pressure exiting the ozone generator is forced through small apertures supported under the water. With negative pressure devices, ozone gas is drawn into the flowing water stream to be treated. Under these mixing conditions, bubbles are sheared and mixed thoroughly with the aqueous fluid, decreasing the liquid film thickness but increasing both interfacial area and contact time. Faster ozone mass transfer rates result in faster disinfection and (usually) oxidation rates. However, slow-to-oxidize organics are unaffected by increased ozone mass transfer rates, and advanced oxidation techniques should be considered for these types of materials.

Ozone transfer efficiencies vary with the number of contacting stages and typically are above 90%. However, since even a 95% ozone absorption efficiency can result in a contactor off-gas containing as much as 740 ppm (by wt) of ozone (based on 1.5 wt-% ozone in air feed gas), treatment is required to reduce the ozone concentration to an acceptable maximum level for discharge to the local environment. This can be accomplished thermally and/or by catalytic means, and sometimes (for low concentrations and/or small volumes) by passage through wet granular activated carbon beds.

Application in the Gas Phase

Reaction rates of contaminants in air are much lower (slower) in the gas phase than in aqueous phase. Additionally, the option of adjusting pH is not open in air spaces, although increasing the relative humidity is an effective option. Pumping ozone gas from the generator into an air space to be treated is the simplest approach, and is most effective when the air contaminants to be treated are rapidly reactive with ozone – e.g., many odoriferous compounds such as hydrogen sulfide, molds, spores, and some airborne microorganisms. For those air contaminants that are only slowly affected by ozone, the accepted procedure is to draw contaminated air into an enclosed structure in which ozone is mixed with the contaminated air for such period of time as is necessary to destroy (or inactivate) the contaminants.

Application of Adjuncts With Ozone

With the advent of ozone advanced oxidation, there are recent instances reported of coupling ozone with either hydrogen peroxide or with ultraviolet radiation, techniques that are designed to promote the formation of hydroxyl free radicals with the stated objective of increasing the amount of microbiocidal activity above that of ozone itself. However, all information developed to date indicates that the half-life of hydroxyl free radicals in water is only microseconds in length, and that the maximum concentration of hydroxyl free radicals that has been measured in aqueous solution is very low, on the order of 10-12 M. These facts tend to indicate that there can be no microbiocidal benefit of hydroxyl free radical over that provided by
molecular ozone. Nevertheless some recent reports indicate what appears to be a synergy – or an increased amount of microbial inactivation by applying these combinations to certain foodstuffs over what is obtained by applying ozone or UV radiation alone. For example, Naitoh, 1992 investigated synergistic sporicidal activities of gaseous ozone and UV irradiation.

**Ozone + Hydrogen Peroxide**

With this combination of oxidants in aqueous solution, both agents destroy each other, and both agents give rise, eventually, to hydroxyl free radicals. It is customary procedure to add the requisite amount of hydrogen peroxide to solution and then pass that solution through an ozone contacting apparatus. Ozone reacts immediately with hydrogen peroxide in solution, and if the amount of ozone dosed in the contactor is always greater than the amount of peroxide initially added to solution, at the outlet of the contact chamber it will not be possible to measure a level of residual ozone in solution. In advanced oxidation practice, it has been learned that for optimum oxidative performance, each pollutant that needs to be destroyed requires a specific weight ratio of peroxide to ozone. It is advisable when evaluating the use of this advanced oxidation process to first determine which polluting constituents of the water or waste water requiring treatment are present, and then to determine experimentally the optimum range of peroxide to ozone weight ratio required for their destruction. Glaze et al., 1987 have shown that if the weight ratio of peroxide to ozone rises above 1:1, the rates of oxidation of organics in water actually slow down. This means that if excess hydrogen peroxide is present over the amount of ozone added, at least some of the advantages of advanced oxidation are lessened. It also means that there will be no molecular ozone present at any time during ozone contacting for microbial disinfection.

**Ozone + UV Radiation**

With this combination of agents, it is customary to place a UV bulb (or multiple bulbs) in the ozone contacting chamber. As water flows through the chamber, first the UV bulb(s) is(are) turned on and ozone is added. As long as the amount of UV radiation dosed is in excess of the amount of ozone present, all ozone will be converted instantaneously to decomposition products, ending rapidly as hydroxyl free radicals.

Despite the theories and the work of Kruith of and Kamp (1999), several reports have been made of an apparent increase in antimicrobial activity in some food applications when ozone is combined in water with either peroxide or UV radiation.

**Advantages and Limitations**

The advantages and limitations of ozone disinfection methods proposed for fresh-cut organic vegetables, fruits and meat products applications (Olmez and Kretzschmar, 2009) are as follows.

**Advantages of ozone treatment**

- High antimicrobial activity compared to non-oxidative biocins (chlorine) in terms of concentration and time.
- Short contact time for disinfection compared to other disinfection methods.
- No residue problem as it is completely utilised and get reduced.
- Non-hazardous at low ppm (lower than 4 ppm) and effective in bactericidal uses.
- No need to store hazardous substance compared to other sanitation methods.
- Lower running costs, cost matters only to filling of oxygen cylinders and power supply.
- No heat requirement and no heat generation in treatment(applicable to heat sensitive foods) &thus saves need of input energy.
- Saves transport of disinfectant chemicals& storing of gas cost.
- Eco-friendly and economically feasible technology.

**Limitations of ozone treatment**

- Ozone is toxic; when inhaled it cause throat and nasal problems, even lead to asthma.
- Ozone is highly unstable gas so controlled release on requirement is to be established.
- Recontamination problems in clean in process pipes as ozone decomposes completely within a short duration.
- Corrosive at high ppm (higher than 4 ppm), care should be taken in using ozone and releasing to treatment chamber.
- It requires regular monitoring in indoor applications for any leakages.
- Higher initial investment for the generation equipment.
- Onsite generation is required as it is unstable.
and not suitable for storage

- Storage of ozone is not possible as it decomposes quickly
- It can be mostly surface treatment as ozone decomposes in short time and it is liable to oxidation with organic matter

Factors Affecting Efficacy of Ozone Processing

There are different parameters that affect the disinfection ability of ozone in liquid processing treatment. Extrinsic and intrinsic parameters that affect the ozone efficacy include flow rate, ozone concentration, temperature, pH, and presence of solid contents (organic matter).

Extrinsic Parameters

Flow Rate

Depending on the gas flow rate applied for ozone production, different bubble sizes are produced. Bubble size has been shown to have an effect on ozone’s solubilisation rate and disinfection efficacy. Ahmad and Farooq, 1985 reported that ozone mass transfer and disinfection efficacy increased as bubble size decreased (ozone bubble size was varied while all other factors were kept constant). The higher interfacial area available for mass transfer at the smaller bubble size may have been responsible for this effect. Decreasing the bubble diameter from 1 cm to 0.1 cm increases the contact area by 32 times (Ogden, 1970). Free suspended bacteria migrate toward the ozone bubbles due to their surface active properties and are preferentially inactivated by comparatively high ozone concentrations at the gas liquid interface of the bubble (Hill and Spencer, 1974).

Concentration

Ozone concentration present or available in the medium is another parameter determining ozone efficacy. Increased ozone concentration causes saturation and thus makes addition of further ozone to the reactor ineffective, resulting in longer times to achieve the same log-reduction values.

Temperature

Ozone solubility in water is 13 times that of oxygen at 0-30°C and it is progressively more soluble in colder water (Rice, 1986). The solubility ratio for ozone increases as the temperature of water decreases (Bablon et al., 1991). As temperature increases, ozone becomes less soluble and less stable, with an increase in the decomposition rate (Rice et al., 1981). The mass transfer of ozone gas into the liquid phase is also influenced by temperature and pH. The inactivating capabilities of ozone are in line with the decreasing temperature (Farooq et al., 1977).

Intrinsic Parameters

pH

The effect of pH on ozone inactivation is mainly attributed to the fact that ozone decomposition rate changes substantially with changes in pH (Farooq et al., 1977b; Roy et al., 1980). Patil et al., 2010a observed that ozone inactivation of E. coli was much faster at the lower pH. The ozone treatment duration required for achieving a 5-log reduction was 4 min at the lowest pH and 18 min at the highest pH studied.

Organic Matter

Ozone demand can be caused by certain organics, inorganics, or suspended solids. Dissolved organic matter reduces the disinfection activity by consuming ozone to produce compounds with little or no microbiocidal activity, thereby reducing the concentration of active species available to react with microorganisms. Williams et al., 2005 studied the inactivation of E. coli in orange juice, and found that the efficacy of ozonation was reduced in the presence of ascorbic acid and organic matter. In wastewater, proteins, carbohydrates, lipids, and organic amines will elevate the concentration of dissolved organic carbon. Oxidizing disinfectants like ozone will lose bacteriocidal strength through reaction with organic matter. The reaction products will generally have weak or no bacteriocidal activity.

Microbial Inactivation by Ozone

Microbial load of raw material, improper handling and storage, use of contaminated wash water, processing equipment, and transportation facilities, as well as cross-contamination from other products contribute to the microbial hazards associated with meat, fruits and vegetables. Ozone destroys microorganisms by the progressive oxidation of vital cellular components. The antimicrobial activity of ozone is based essentially on its powerful oxidizing effect, which causes irreversible damage to the fatty acids in the cell membrane and to cellular macromolecules, such as proteins, and DNA (Fettner and Ingols, 1959;
This action is particularly effective in air at high relative humidity, the bacteria being killed by ozone more readily in the swollen state than when dry. The bacterial cell surface has been suggested as the primary target of ozonation. Microorganisms are inactivated by disruption of the cell envelope or disintegration leading to cell lysis.

Two major mechanisms of ozone destruction of the target organisms were identified:

1. Ozone oxidizes sulfhydryl groups and amino acids of enzymes, peptides and proteins to smaller peptides
2. Ozone oxidizes polyunsaturated fatty acids to acid peroxides (Victorin 1992).

Some authors concluded that molecular ozone is the main inactivator of microorganisms (direct oxidation), while others emphasize the antimicrobial activity of the reactive by-products of ozone decomposition (indirect oxidation) such as OH, O₂⁻, and HO₂ (Chang 1971; Harakeh and Butler 1985; Glaze and Kang 1989; Hunt and Marinas 1997). Both molecular ozone and the free radicals produced by ozone breakdown play a part in this inactivation mechanism but there is no consensus on which is more decisive. It has not been well established whether molecular ozone or the radical species are responsible for inactivation of microorganisms. Thus there are two main reactions happening on incidence of ozone on the microbial surfaces viz. direct and indirect reactions. Ozone takes any of the pathway or both for the oxidation of sulfhydryl group of enzymes, amino acids of peptides, proteins and enzymes and polyunsaturated fatty acids.

Direct reaction

Direct reaction with molecular ozone, is the predominant mechanism for inactivation of microorganisms (Finch et al., 1992; Labatiuk et al., 1994; Hunt and Marinas, 1997). It is the direct oxidation of target groups in microbial cell by ozone. It is likely that the relative importance of direct and indirect reactions with ozone in determining microbial inactivation responses will vary between microorganisms (Blatchley and Hunt, 2002). Because of the molecular structure of ozone, it can act as an electrophilic or nucleophilic agent during reactions (von Gunten, 2003), with these types of reaction occurring in solutions containing organic pollutants (with microbes). Generally, electrophilic reactions will occur with organic water contaminants with a high electron density and will act faster in solutions consisting of high levels of aromatic compounds (Gottschalk et al., 2010). Nucleophilic reactions take place mainly when there is a shortage of electrons and particularly at carbon compounds that contain electron-withdrawing groups such as –COOH and –NO₂. However, for these groups the reaction speed is much lower. Overall, the direct oxidation of organic matter by ozone involves a quite selective reaction mechanism (von Gunten, 2003). Moreover, it is important to note how the pH value of the water system can influence ozone decomposition; with pH > 7 causing an increase in the rate of ozone decomposition. Also, in strongly acidic solutions (pH < 3) the OH radicals do not influence the decomposition of ozone.

Indirect reaction

Indirect reactions with radicals are responsible for inactivation for some groups of microbes (Bancroft et al., 1984). Ozone decomposition has been explained as occurring in three stages; initiation, promotion and inhibition. During the initiation step, free radicals are generated, such as superoxide radical ions and hydroperoxide radicals, which lead to formation of the highly reactive hydroxyl radical. These hydroxyl radicals are one of the factors contributing to ozone decomposition. The promotion step involves regeneration of the hydroperoxide and superoxide radicals through reactions involving participation of promotors such as formic acid, glyoxylic acid, primary alcohols and aryl groups. In contrast, in the inhibition step the consumption of hydroxyl radicals occurs via ions like bicarbonate, carbonate, tertiary alcohols and alkyl groups, without regeneration of the superoxide radical ion (Staehelin and Hoigné, 1985; Khadre et al., 2001). Bicarbonate ions are generally present in microbial cells, which could act as scavengers of radicals otherwise responsible for inactivation of microorganisms.

Additionally, factors promoting ozone decomposition in the system can lead to faster dissipation of ozone, resulting in a requirement for increased ozone concentration in order to achieve the desired inactivation level (Zuma et al., 2009). The resultant disruption or lysis of cell walls (probably by oxidative destruction) associated with ozone is a faster inactivation mechanism than that of other disinfectants, which require the disinfecting agent to permeate through the cell membrane in order to be effective (Pascual et al., 2007). Scott and Lesher (1963) reported that ozone caused
leakage of cell contents into the medium and lysis of some cells. Therefore, ozone-demanding substances are generated during the ozone inactivation process. Finch and others (1988) found that E. coli cells demanded 0.06 mg/L ozone after lysis and attributed the second phase of inactivation to this ozone-created demand (Kim and Yousef, 2000). Generally, with regard to the spectrum of microbial action, each microorganism has an inherent sensitivity to ozone. Bacteria are more sensitive than yeasts and fungi. Gram-positive bacteria are more sensitive to ozone than Gram-negative organisms, and spores are more resistant than vegetative cells. Due to the mechanism of ozone action, which destroys the microorganism through cell lysis, the development of resistance to ozone disinfection is not found (Pascual et al., 2007).

Target sites of ozone activity

Inactivation of bacteria by ozone is a complex process because ozone attacks numerous cellular constituents including proteins, unsaturated lipids and respiratory enzymes in cell membranes, peptidoglycans in cell envelopes, enzymes and nucleic acids in the cytoplasm, and proteins and peptidoglycan in spore coats and virus capsids.

Cell envelope

Ozone may oxidize various components of cell envelope including polyunsaturated fatty acids, membrane-bound enzymes, glycoproteins and glycolipids leading to leakage of cell contents and eventually causing lysis (Scott and Lesher, 1963; Murray et al., 1965). When the double bonds of unsaturated lipids and the sulphydryl groups of enzymes are oxidized by ozone, disruption of normal cellular activity including cell permeability and rapid death ensues. Dave (1999) found that treatment of Salmonella enteritidis with aqueous ozone disrupted the cell membranes as seen in transmission electron micrographs. However, Komanapalli and Lau (1996) found that short-term exposures of E. coli K-12 to ozone gas compromised the membrane permeability but did not affect viability, which progressively decreased with longer exposure.

Bacterial spore coat

Foegeding, 1985 found that bacterial spores (Bacillus cereus) with coat proteins removed were rapidly inactivated by ozone, compared to intact spores. The researcher concluded that the spore coat is a primary protective barrier against ozone. Recently, Khadre and Yousef, 2001 found that spores of Bacillus subtilis treated with aqueous ozone showed heavily disrupted outer spore coats. Transmission Bacillus spores treated with ozone suggest that ozone inactivates spores by degrading the outer spore component (spore coat layers make up approximately 50% of the spore volume), thus exposing the cortex and core to the action of ozone (Foegeding, 1985; Khadre et al., 2001). Young and Setlow, 2004 determined that ozone does not kill spores by DNA damage but rather by damaging the ability of the spores to germinate. The researchers hypothesised that damage to the inner membrane of spores causes defects in spore germination.

Enzymes

Several authors referred to enzyme inactivation as an important mechanism by which ozone kills cells. Sykes, 1965 reported that chlorine selectively destroyed certain enzymes, whereas ozone acted as a general protoplasmic oxidant. Ingram and Haines, 1949, on general destruction of the dehydrogenating enzyme systems in the cell, proposed that ozone kills E. coli by interfering with the respiratory system. Takamoto and others, 1992 observed that ozone decreased enzyme activity in E. coli at a greater degree in case of cytoplasmic â-galactosidase than in case of the periplasmic alkaline phosphatase. Inactivation of enzymes by ozone is probably due to oxidation of sulphydryl groups in Cysteine residues (Chang 1971).

Nucleic material

Reaction of aqueous ozone with nucleic acids in vitro found that it may damage nucleic material inside the cell. Ozone modified nucleic acids in vitro, with thymine being more sensitive than cytosine and uracil (Scott, 1975; Ishizaki et al., 1981). In another study, ozone opened the circular plasmid DNA and reduced its transforming ability, produced single double-strand breaks in plasmid DNA (Hamelin, 1985), and decreased transcription activity (Mura and Chung 1990). Herault and Chung, 1984 found that ozone may induce mutations. Compared to other known mutagens, ozone was found to be a weak mutagen on Saccharomyces cerevisiae (Dubeau and Chung, 1982).

Ozone reactions against virus

Sproul and Kim, 1980 found that aqueous ozone inactivated bacteriophages (f2 and T4) by
attacking capsid protein, with liberation and inactivation of the nucleic acid. The RNA from bacteriophage (f2) was partially inactivated prior to release from the capsid. They suggested that ozone breaks the protein capsid into subunits liberating RNA and disrupting virus adsorption to the host pili, and that the RNA may be secondarily inactivated. The DNA released from bacteriophage (T4) was rapidly inactivated by ozone at about the same rate as that in the intact phage. CK Kim et al., 1984 found that ozone randomly destroyed the head, collar, contractile sheath, end plate, and tail fibers and liberated the DNA from the head. Yoshizaki and others, 1988 and Shriniki and others, 1988 concluded that the major cause of tobacco mosaic virus (TMV) inactivation by ozone was the inability of the treated virus to uncoat. Roy and others, 1981 found that ozone altered two of the four polypeptide chains in the poliovirus protein coat. They, however, attributed the inactivation of the virus to the damage in its RNA by ozone. The observation by Herbold and others, 1989 confirmed the hypothesis that damage to viral envelopes is the main cause of inactivation of viruses by ozone. Enveloped viruses such as HIV are expected to be much more resistant to ozone compared to non-enveloped viruses such as poliomyelitis.

Objectives of Ozone in Food Industries

Ozone is an accepted commercial technology in many aspects of the foods industry, ranging from irrigation and soil treatment (Parmenter et al., 2004), to spraying crops to avoid spraying noxious chemicals (Steffen and Rice, 2008), odour control in animal housing (Parmenter et al., 2004) and for uses in food processing plants such as water and air treatment, food decontamination and disinfection, safe packaging and storage etc. In general, ozone finds wide application in the food industry, including surface decontamination of meat, fruits and vegetables, drinking water disinfection, pesticide removal, safe storage and wastewater treatment (Guzel-Seydim et al., 2004; Karaca and Velioglu, 2007).

Ozone is applied in either gaseous or aqueous form. Ozone is very effective against bacteria because even concentrations as low as 0.01 ppm are toxic to bacteria. Whereas disinfection of bacteria by chlorine involves the diffusion of HOCl through the cell membrane, disinfection by ozone occurs with lysing (i.e., oxidative rupture) of the cell wall. Disinfection rates by ozone, however, depend on the type of organism and are affected by ozone concentration, temperature, pH, turbidity, the presence of ozone-oxidizable materials, the tendency (or not) for the microorganisms to form clumps, and the type of ozone contact or employed (Zhu et al., 1989). The presence of ozone-oxidizable substances in water exerts an ozone demand, and this can retard disinfection until the initial ozone demand has been satisfied, at which point rapid disinfection is observed. Its efficacy against a wide range of microorganisms, including bacteria, fungi, viruses, protozoa and bacterial fungal spores, has been reported (Restaino et al., 1995; Khadre et al., 2001; Cullen et al., 2009). Such advantages make ozone attractive to the food industry and consequently it has been affirmed as Generally Recognised as Safe (GRAS) for use in food processing (Graham, 1997) and was approved as an antimicrobial food additive in 2001 (FDA, 2001). Several incidents of food borne disease have been associated with fruit and vegetable products.

Some of the ozone uses in food industries as given by Bharathraj (2000) are as follows.

Removal of contaminants

Fungicides, pesticides and other chemicals use during farming practices can contaminate the surface of food. This can be potentially dangerous as simple washing cannot remove these accumulations. Ozone can be used to oxidize the chemicals and remove the contaminate safe for sale or for further processing.

Cleaning in process (CIP)

Ozone charged water is used for CIP for cleaning pipes, tanks, floors, surface equipment etc. Use of ozone system to food processing is that it provides the opportunity to reuse the water that could bring about a lot of savings in terms of availability and water cost. CIP washing of plant processing equipment and drains with ozone-containing water is now common practice (Parmenter et al., 2004; Lowe, 2002).

Sanitation

Food for consumption must be free from pathogenic microorganisms. Contamination can occur from harvesting stage, during transportation, from processing water, equipment or from human interventions or by cross contamination. Usually non-oxidative biocides such as chlorine were widely used for 2-log unit reduction of microorganisms. Chlorine, the most common used disinfecting agent, selectively destroys certain intracellular enzyme
systems, whereas ozone causes widespread oxidation of internal cellular proteins causing rapid cell death. Chlorine, however, is not effective for virus. Also it takes more concentration and exposure time for microbial reduction as compared to ozone since the mechanism is by penetration through the membrane. Ozone is an oxidative biocide and is better than non-oxidative to avoid undesirable taste or carcinogenic effects. Ozone is 3000 times powerful than chlorine and regarded as Generally Recognised as Safe (GRAS). Commercial processing applications of ozone expanded in processing water treatments in the near future especially in fish hatcheries (Blogoslawski et al., 1993; Brazil and Summerfelt, 2005), beverage-producing plants, and wineries (Steffens, 2006). The use of ozone for the sanitisation of equipment and surfaces in the beverage manufacturing industry has yielded impressive results in terms of controlling microorganisms and saving costs due to less chemical handling and less maintenance (Clear Water Tech, 2002; Hampson, 2000; Tinney, 2002).

Stop ripening and spoilage

Consideration of fresh food requirement standards for consumers should assure fresh and safe food products. Ethylene formation on the food surface is responsible for the food ripening and spoilage. By virtue of its chemical properties, ozone prevents ethylene formation and thereby retards ripening and spoilage by microorganisms. This property extends the shelf life of food.

Ozone oxidises ethylene completely and leaves carbon dioxide, water and oxygen. The reaction is as follows.

$$C_2H_4 + 6O_3 \rightarrow 2CO_2 + 2H_2O + O_2$$

Cold storage

Utilisation of the properties makes ozone eminently suitable for increasing the storage life of perishables foods in refrigerated premises. At the same time, it is economic as the investment and operational cost of the equipment are on an acceptable level in relation to the size of refrigeration rooms. Its application eliminates the risk of unpleasant odours or other traces of antiseptics used for preservation of food stuffs. During storage ozone exerts a threefold effects by destroying the microorganisms, oxidising odours and affecting the processes of metabolism. Its primary action is mold free surface and has only slight depth of penetration.

Increasing the moisture content of the environment favourably influences the germicidal effect. This brought about by the swelling of microbes making them more susceptible to destruction. Experiments conducted with beef showed that ozone is most efficient if the surface has a definite moisture content of around 60%.

Current Research and Applications in Foods

Consumer preference for minimally processed foods and foods free of chemical preservatives, as well as recent outbreaks of food borne pathogens, identification of new food pathogens have all stimulated demand for novel food processing and preservation systems Minimising the occurrence of pathogenic and spoilage microorganisms in fruits, vegetables and their products is a primary food-safety concern. For this purpose, the incorporation of ozone (by itself or in combination with other technologies) resulted either in improved product quality, significant costs savings, or both.

Ozone in seafood and shell fish processing

Violle, 1929, Salmon and LeGall, 1936, Fauvel et al., 1979 developed the use of ozone for shell fish depuration from laboratory stage to full commercial installations in Southern Europe. Depuration is a process whereby shellfish, freshly harvested from their natural environments, are placed for several days in storage chambers, through which clean, pathogen-free water is passed. Ozonated water was used for depuration. Over a several day time period, the molluscs cleanse themselves by passing disinfected water through their systems, thus eliminating pathogenic microorganisms imbibed from their natural environments.

Abatement of ethylene by ozone

Potential storage life of citrus fruits with fairly good appearance and eating quality can be obtained if fruits are stored under the most optimum conditions after harvest. Ethylene, also known as stress hormone, has a special role in fruit humidity, ripening and senescence, and therefore has its own importance in postharvest management of citrus. Citrus fruits have a very low rate of ethylene evolution, in the amount of 0.1 mL/kg/h. Even this rate can slowly build up ethylene concentration in closed chambers (Ladaniya, 2007). Ozone has shown a potential to meet this criterion and given
encouraging results. Skog and Chu, 2001 claimed that ozone could reduce the level of ethylene in the air in a cold storage room. Indeed, ozone was found to be effective in removing ethylene from export containers (Palou et al., 2001).

Many factors such as freezing, drying or high carbon dioxide concentrations can increase ethylene evolution from agricultural products. Controversial results are found in the literature about ozone from this point of view. Actually, ozone is known to impose oxidative stress and cause many physiological changes, including ethylene synthesis in crops (Forney, 2003).

During ozone exposure, plants attempt to maintain a constant redox potential in their cells. In many cases, this result in an increase in the concentrations of antioxidants enzymes and compounds that play a major role in the defense system of the plants against oxidative stress. Ascorbic acid levels in spinach (Luwe et al., 1993), anthocyanins in blackberries (Barth et al., 1995) and concentrations of phytoalexins in grapes (Sarig et al., 1996) were reported to increase after ozone exposure. More research is needed to identify and define the physiological responses of each commodity to ozone.

**Microbial reduction and sanitation by ozone**

Ozone destroys microorganisms by the progressive oxidation of vital cellular ozonation. Numerous studies were conducted to investigate the effectiveness of ozone against various kinds of microorganisms. Studies on microbial inactivation by ozone in fruits and vegetables are summarized in Table 1. Besides microbial inactivation, ozonation has also been applied for the purpose of mycotoxin degradation in food products.

**Ozone in fluid food processing**

Microbial studies to date typically show mandatory 5-log reductions of spoilage and potentially pathogenic species most commonly associated with fruit and vegetable juices achieved using ozone. Applying ozone at doses leading to effective decontamination may impact the sensory qualities of food. Ozone is not universally beneficial and in some cases may promote oxidative spoilage in foods. Oxidation of undesirable or unwanted organic and inorganic compounds (iron, manganese, nitrite, cyanide, hydrogen sulphide) by application of ozone is rapid (Rakness, 2005). Dock, 1995 reported no detrimental change in quality attributes of apple cider when it was treated with ozone. Segovia Bravo et al. (2007) concluded that ozone treatment (7g /h) for 24 h caused rapid destruction of most of the polyphenols present in green table olive solutions and ozone bubbling for a further 72 h was necessary to reduce the remaining tyrosol content in the solution.

**Ozone in meat processing**

Steffen and Rice, 2010 demonstrated the use of ozone (in gas and aqueous phases) for the preparation of complete meals in a central food processing plant; the meals were then packaged and sealed in a sterilised manner. Ozone is reported to be effective against contamination of natural casings, which are generally contaminated with several microorganisms. Benli et al., 2008 suggested that a combination of treatments, such as washing with ozonated water to whiten casings with another treatment, such as irradiation, might prove effective for enhancing the casing value while ensuring the destruction of potential food borne pathogens.

Reagan et al., 1996 evaluated trimming techniques and beef carcass washing techniques to improve the microbial quality of meat. Intervention treatments included knife trimming, washing with water and rinsing with ozone (0.3–2.3 ppm) or hydrogen peroxide (5%). Ozone treatment reduced carcass surface contamination by 1.30 CFU/cm², whereas hydrogen peroxide reduced aerobic plate counts by 1.14 CFU/cm². Greer and Jones (1989) evaluated the impact of gaseous ozone treatment on beef carcass bacterial spoilage profiles and on meat quality and carcass shrinkage. They found that psychrotrophic bacterial growth was retarded on carcass surfaces while under ozone atmosphere.

Ozone has also been used as a pre-treatment before cooking to determine any synergistic activity on reducing microorganisms. Novak and Yuan (2004a) treated beef surfaces with ozone then cooked the treated beef at temperatures of 45–75 °C to determine the impact on enterotoxin-producing strains of Clostridium perfringens. The authors reported a 1–2 log CFU/g reduction in C. perfringens as a result of aqueous ozone treatment and heating at 45–75 °C. Additionally, they reported a reduction in spore count with the same treatments, but the magnitude of reduction was very small, indicating that the spores were much more resistant
Table 1. Current researches published on microbial inactivation by ozone

<table>
<thead>
<tr>
<th>Product</th>
<th>Treatment</th>
<th>Findings</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Orange and Lemon</td>
<td>Gas ozone (0.3ppm) for 4 weeks</td>
<td>Spore was reduced of P. Italicum</td>
<td>Paluo et al., 2001</td>
</tr>
<tr>
<td>Peach</td>
<td>Ozone atmosphere storage (0.3ppm, 5°C) for 4 weeks</td>
<td>Aerial mycelial growth and sporulation were inhibited</td>
<td>Paluo et al., 2002</td>
</tr>
<tr>
<td>Strawberry</td>
<td>Storage for 3 days at 2°C with 1.5ppm ozone</td>
<td>Mycelial growth developed more slowly</td>
<td>Nadas et al., 2003</td>
</tr>
<tr>
<td>Apple</td>
<td>Ozone bubbling and dipping in pre-ozonated water, 3min</td>
<td>Decrease in counts of E col (&lt;1 log CFU)</td>
<td>Achen et al., 2001</td>
</tr>
<tr>
<td>Citrus</td>
<td>Basket immersed in ozonated water at 1.5-10ppm, stored for 7-21 days at 10°C and 20°C</td>
<td>5 ppm for 5 min reduced aerobic bacteria population</td>
<td>Smilanick et al., 2002</td>
</tr>
<tr>
<td>Celery</td>
<td>Dipping to (0.03,0.08,0.18 ppm) ozonated water for 5 min and storing at 4°C for 9 days</td>
<td>Populations of total bacteria reached to 5.72, 5.64 and 5.63 from 5.08 log CFU/g</td>
<td>Zhnag et al., 2005</td>
</tr>
<tr>
<td>Lettuce</td>
<td>Soaking in ozonated water with 5 ppm ozone</td>
<td>Reduced aerobic bacteria and yeast within 10 min</td>
<td>Koseki et al., 2001</td>
</tr>
<tr>
<td>Orange juice</td>
<td>Ozone gas pumped into juice</td>
<td>E. coli was reduced to 5 log cyle, Ascorbic acid decreased</td>
<td>Angelino et al., 2003, Williams et al., 2004</td>
</tr>
<tr>
<td>Cheese</td>
<td>Gaseous ozone at 3 different levels</td>
<td>Reduction of fungal counts on ripening room and on cheese surface</td>
<td>Serra et al., 2003; Pinto et al., 2007</td>
</tr>
<tr>
<td>Orange and lemon</td>
<td>Ozone gas (0.1-2g/m³) at 4.5-10°C</td>
<td>Decrease of P. digitatum and its sporulation</td>
<td>Palou et al., 2001</td>
</tr>
<tr>
<td>Barley</td>
<td>Gaseous ozone</td>
<td>Reduction mycelia grow and spores germinates</td>
<td>Allen et al., 2003</td>
</tr>
<tr>
<td>Maize</td>
<td>Gaseous ozone</td>
<td>Reduction of 63% fungal growth counts after 3 days</td>
<td>Kells et al., 2001</td>
</tr>
<tr>
<td>Dried fig</td>
<td>0.01-0.02g/m³ gaseous ozone for 3-4 hours</td>
<td>Reduction of fungal counts (mycoflora)</td>
<td>Ortekien et al., 2006</td>
</tr>
<tr>
<td>Onion</td>
<td>Gaseous ozone</td>
<td>Reduction on spore germination, change of colony colour (Aspergillus)</td>
<td>Vijayanandraj et al., 2006</td>
</tr>
<tr>
<td>Pea seed</td>
<td>3.85 g/m³ of gaseous ozone for 7.5,15 &amp; 30 minutes</td>
<td>Reduction of fungal counts (Fusarium, Alternatia)</td>
<td>Ciccarese et al., 2007</td>
</tr>
<tr>
<td>Wheat</td>
<td>1.4 g/m³ gaseous ozone</td>
<td>Reduction of fungal counts (Micromycetes)</td>
<td>Raila et al., 2006</td>
</tr>
<tr>
<td>Tomato</td>
<td>Washing with ozonated water (3.8ppm) for 10 min</td>
<td>Spores of B. cinerean on the surface were inactivated</td>
<td>Ogawa et al., 1990</td>
</tr>
<tr>
<td>Blackberry</td>
<td>Storage for 12 days at 2°C in an ozone atmosphere</td>
<td>Suppressed fungal development for 12 days</td>
<td>Barth et al., 1995</td>
</tr>
<tr>
<td>Date fruit</td>
<td>0.002, 0.006, and 0.01 g/m³ gaseous ozone for 1 hour</td>
<td>Reduction of fungal counts (total mycoflora)</td>
<td>Najafi and Khodaparast, 2009</td>
</tr>
</tbody>
</table>

to ozone and thermal treatments. The authors concluded that ozone treatment followed by heat treatment allowed reductions at cooking temperatures that normally would not impart the reduction by themselves.

Ozone in grain processing

The control of pest (insects and microorganisms including mould, fungi and bacteria) development in stored grains after harvest is essential as it currently leads to a grain yield loss around 3–10% in developed countries, and can reach 50% in certain countries (Jayas, 1999; Fleurat-Lessard, 2004; Magan and Aldred, 2007).

Storage grains are very susceptible to a number of insects, such as Tribolium, Sithophilus and moths, which cause considerable damage and
which could potentially develop resistance to the currently used insecticides. Ozone that can be used in fumigation is an interesting alternative to applied chemicals for the control of insect development. Kells et al. (2001) evaluated the efficiency of ozone fumigation in a corn grain mass against adult insects, such as the red flour beetle (Tribolium castaneum), maize weevil (Sithophilus zeamais) and larvae from the Indian meal moth (Plodia interpunctella). Insects were put in cages containing maize kernels and placed into a column filled with grains and positioned just below the surface. The columns were treated with or without ozone (50 ppm for 3 days or 25 ppm for 5 days) and the number of dead insects was determined. Results demonstrated a significant insect mortality increase (92–100% compared to 3–10% in the control) when the insect species in grain samples were treated with 50 ppm for 3 days. The lower dose was also significantly efficient but led to a lower insect mortality (77–99.9% depending on the insect species). Similar results were obtained by Maier et al., 2006 using identical conditions but with insects positioned deeper in corn grain samples (0.6 m below the grain surface) and in the plenum of silos.

Due to its inactivating action on fungi, ozone can also be considered as helping to reduce mycotoxin accumulation during grain storage. Furthermore, its oxidant properties could also be used for mycotoxin degradation and detoxification. Degradation of aflatoxin (McKenzie et al., 1997), as well as trichothecenes (Young et al., 2006), is initiated by the attack of a double bond with addition of two oxygen atoms, which further leads to the molecule breaking apart. Young et al., 2006 furthermore pointed out that trichothecene degradation depends on ozone concentration as well as on pH.

Effect of Ozone on Product Quality and Nutrition

Microbial studies to date typically show that the mandatory 5 log reductions of spoilage and potentially pathogenic species most commonly associated with fruit and vegetable or juices may be achieved. A number of studies report the effects of ozone on quality parameters of treated fruits and vegetables (Zhang et al., 2005; Fonseca and Rushing, 2006). The effects of ozone treatment on quality and physiology of various foods are reported. Applying ozone at doses that are large enough for effective decontamination may change the sensory qualities of food. Ozone is not universally beneficial and in some cases may promote oxidative spoilage in foods. Surface oxidation, discolouration or development of undesirable odours may occur in substrates from excessive use of ozone (Khadre et al., 2001). Dock, 1999 reported no detrimental change in the quality attributes of apple cider when it was treated with ozone.

Chemical attributes

No change in onion chemical composition and sensory quality was reported by Song et al., 2000. Ozone-containing water treatment resulted in no significant difference in total sugar content of celery and strawberries (Zhang et al., 2005) during storage. Ozonation is expected to lead to the loss of antioxidant constituents, because of its strong oxidising activity. However, ozone washing treatment was reported to have no effect on the final phenolic content of fresh-cut iceberg lettuce (Beltrán et al., 2005a). Contradictory reports were found in the literature regarding ascorbic acid. Decomposition of ascorbic acid in broccoli florets was reported after ozone treatment by Lewis et al., 1996, but Zhang et al., 2005 reported no significant difference in ascorbic acid content between treated and non-treated at other concentration.

Visual quality

Gabler et al., 2010 investigated the efficacy of ozone in controlling post-harvest decay of table grapes and for the potential replacement of sulfur dioxide, which is used as a commercial fumigant. They observed that ozone fumigation with up to 10 000 l/L for up to 2 hours helps to control postharvest grey mould of table grapes caused by Botrytis cinerea. However, grapes stored in ozone-rich atmospheres may develop thin longitudinal darkened lesions. This injury is reported to be irregular and was not always associated with an ozone dose or cultivar (Gabler et al., 2010). Martínez-Sáñez et al., 2006 investigated the effect of several sanitisers on the visual quality and colour of rocket leaves during storage in air and low O₂ (1–3 kPa) + high CO₂ (11–13 kPa) for 15 days at 4 °C. They observed that ozone effects were comparable with other sanitisers, except for lactic acid treated samples.

Texture

Texture or firmness is an important rheological property pertinent to fresh fruits and
vegetables. Fruits and vegetables with a firm, crunchy texture are highly desirable because consumers associate these textural attributes with freshness and wholesomeness. The appearance of a soft or limp product may give rise to consumer rejection prior to consumption (Rico et al. 2007). Textural changes in fruits and vegetables could be due to various enzymatic and non-enzymatic processes. Ozone treatment of fresh fruit and vegetables either by washing or in storage consisting of ozone gas is reported to have significant effects on texture. Firmness of fresh coriander leaves was reported to decrease through washing with ozone-containing water compared to control. Another study conducted by Selma et al., 2008 reported no significant changes in firmness of fresh-cut cantaloupe irrespective of gaseous ozone concentration (5000 or 2000 ppm) for 30 minutes during storage compared to control. Change in texture during ozonation and subsequent storage may possibly be due to postharvest changes in cellulose and hemicellulose contents due to ozone application during MAP. This could be due to polymerisation and epimerisation of cellulose and hemicelluloses contents of cell walls inducing thickening of the cell walls, causing textural changes in fresh-cut green asparagus during storage after ozone treatment (An et al., 2007). An et al., 2007 reported an increase in cellulose, hemicelluloses and lignin content during MAP storage after pre-treatment with aqueous ozone.

Ozonation of fruits has been reported to enhance firmness of citrus fruits and cucumbers compared to controls (Skog and Chu, 2001). Ozone is reported to delay softening in strawberries during cold-room storage and storage at room temperature (Nadas et al., 2003). Ozone is a strong oxidising agent which can cause oxidation of feruloylated cross linkages or phenolic cross linkages among cell-wall pectin, structural proteins or other polymers, and thereby change the firmness of the product (Heun Hong and Gross, 1998).

**Sensory quality**

The most notable effect of ozone on the sensory quality of fruits reported in the literature is the loss of aroma. Ozone-enriched cold storage of strawberries resulted in reversible losses of fruit aroma (Nadas et al., 2003; Perez et al., 1999). This behaviour is probably due to the oxidation of volatile compounds. However, Tzortzakis et al., 2007 did not observe any significant changes in tomato fruit weight, antioxidant status, CO$_2$/H$_2$O exchange, ethylene production or in organic acid, vitamin C (pulp and seed) or total phenolic content when exposed to ozone concentrations ranging between 0.005 and 1.0 imol/mol at 13°C and 95% RH. Similar results were reported by Kute et al., 1995 for strawberry exposed to ozone concentrations between 0.3 and 0.7 imol/mol for up to 1 week. Applying ozone at doses that are large enough for effective decontamination may change the sensory qualities of these products.

**Future Trends**

Ozone application has given promising results for important problems of food industry, such as mycotoxin and pesticide residues. Degradation products, formed after ozonation of these residues, have not exactly been determined, and this seems to be the most crucial obstacle on this subject. In vivo and in vitro toxicological tests are needed to be conducted to screen the effects of degradation products on human and animal health. Through emerging new techniques, as well as improvements and innovations in ozone generating and application systems, the subject will be evaluated more effectively in future.

S. Patil and P. Bourke said that the possibility that interaction of ozone with the types of organic material present in the food product results in differing radical production trends that can lead to microbial inactivation should be studied. Therefore, studying the mechanism of the reaction of ozone with organic materials will contribute to establishing the impact of specific radical species on target microorganisms. Further research is required to ascertain the interaction of food constituents with ozone and role of resulting compounds in the inactivation process. Optimization of process parameters may also follow more specific process-related studies on mechanisms of action.

Ozone is an effective sanitizer with great potential applications in the food industry. It decomposes into simple oxygen with no safety concerns about consumption of residual by-product. Due to its high oxidation capacity and microbial inactivation potential, ozone has prevented various kinds of microbial spoilages usually encountered in fruits and vegetables. Decontamination of products by ozone depends on number and kind of contaminating microorganisms, physiology of the product, ozone application system, temperature, pH, and other factors. If
improperly used, ozone can cause some deleterious effects on physiology and quality of products such as losses in sensory quality. For effective and safe use in food processing, optimum ozone concentration, contact time and other treatment conditions should be defined for all products. Pilot trials must be conducted before starting commercial application, because every ozone application is unique.

LITERATURE CITED


Patil, S., Valdravidis, V.P., Cullen, P.J., Frias, J., Bourke, P., 2010a. Inactivation of Escherichia coli by ozone treatment of apple juice at different pH levels. Food Microbiol. 27 (6), 835840.


*Received on 25-07-2015 Accepted on 31-07-2015*