

A Review on Application of Ozone in the Food Processing and Packaging

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Abstract

The use of ozone in the processing of foods has recently come to the forefront as an anti-microbial agent for food treatment, storage and processing. Ozone is now being used as a safe, powerful disinfectant to control biological growth of unwanted organisms in products and equipment used in the food and beverage industries. In liquid solution, ozone can be used to disinfect equipment, process water, and some food products. In gaseous form, ozone helps sanitize and assist in the preservation of certain food products, and is also used to sanitize food packaging materials. Some products currently being preserved with ozone include eggs during cold storage, fresh fruits and vegetables, and fresh fish. In the agriculture industry ozone is being used for disinfection. Another important application is the use of ozone as an alternative to methyl bromide as a fumigant to control insect infestations in stored food, grains and other agricultural products. It is also being used as a general soil fumigant/sterilant in drip irrigation systems. In this review, present status of ozone application in food industry was discussed.

Key words: Ozone, Food industry, Microorganisms, Fruits, Vegetables.

1. Introduction

Ozone, first discovered in 1840, being utilized as a disinfection agent in the production of potable water in France in the early 1900's. The majority of early development was limited to Europe where it became more widely used in drinking water treatment. The potential utility of ozone to the food industry lies in the fact that ozone is 52% stronger than chlorine and has been shown to be effective over a much wider spectrum of microorganisms than chlorine and other disinfectants. Complementing the effectiveness, is the fact that ozone, unlike other disinfectants, leaves no chemical residues and degrades to molecular oxygen upon reaction or natural degradation. The fact that ozone has a relatively short half-life is both an asset and a liability to practitioners.

The preservation of food has been a challenge for mankind throughout the ages. Preservation of food can be defined as the extension of shelf-life of raw materials or prepared foods beyond their natural decay times. The food industry has generally concentrated on inactivating or killing of microorganisms and enzymes as a means of preservation by using a number of physical methods, mostly involving heat (Jakob and Hensen, 2005). Heat, particularly through cooking, has long been the principle method of eliminating

pathogens. New technologies, including steam pasteurization, steam vacuuming, flash pasteurization and others continue to rely on heat to control or reduce harmful microorganisms in food (Majchrowicz, 1999). Many of the products resulting from these processes have become established in the diet throughout the world for decades and are important in own right. However, the application of heat results in products that are radically changed from their fresh counter parts (Leadley and Williams, 2006). Also, the effectiveness of heat processing is dependent on treatment temperature and time. Though thermal treatment helps kill vegetative organisms and some spores, however, the magnitude of treatment-time and process temperature is also proportional to the amount of nutrient loss, development of undesirable flavours and deterioration of functional properties of the food products (Dolatowki *et al.*, 2007). Non-thermal technologies, such as chemical rinsing and others work without heat, affects the composition and cellular activity of pathogens and ultimately killing them (Majchrowicz, 1999). But, the consumers demand for safe (due to mistrust for artificial chemical preservatives) and fresh-like products. The food industry is therefore currently in need of innovative processing technologies in order to meet the

consumer's needs and demands. This has given impetus to research and led to studies for development of alternative processing methods. Hence, a wide range of novel processes have been studied over the last 100 years. Many of these technologies remain much in the research arena; however others have come onto the brink of commercialization (Leadley and Williams, 2006). Attention is now focused on ozone which is a powerful sanitizer that may meet expectations of the industry, approval of the regulatory agencies and acceptance of the consumer (Khadre *et al.*, 2001).

Sanitizers have been used in food processing facilities to control contaminant microorganisms, particularly those causing food borne diseases. However, use of some sanitizers has been limited or banned because of the potential health hazards. On the other hand, the need for potent antimicrobial agent has increased in recent years due to increasing outbreaks and emergence of new food pathogens. Therefore, the food industry is in search of disinfectants that are effective against foodborne pathogens and safe to use in many specific applications of food processing. One such compound is ozone (Kim *et al.*, 1999). Furthermore, research and commercial applications have verified that ozone can replace traditional sanitizing agents and provide other benefits (Cena, 1998; Graham, 1997).

2. What is Ozone?

Ozone (O₃) is an allotropic form of oxygen (O₂), i.e. it is made up of same atoms, but they are combined in different form. The difference is the presence of three oxygen atoms, whereas "common oxygen" has only two. It has low molecular weight (MW = 48) whose three oxygen atoms chemically are arranged in chain. Ozone is then enriched oxygen (O₃) (Guzel-Seydim *et al.*, 2004).

Table 1: Major physical properties of pure ozone

Boiling point	-111.97±0.3°C
Melting point	-192.57±0.4°C
Critical temperature	-12.1°C
Critical pressure	54.6 atm

Source: Guzel-Seydim *et al.* (2004)

Ozone is a gaseous compound which is naturally present in the atmosphere and formed as a result of lightning or high energy UV radiation (Jakob and Hansen, 2005). Ozone has been used for decades for potable water treatment in order to disinfect the water and to help remove foul odour and organic/inorganic impurities (Rice, 1999; Muthukumarappan *et al.*, 2000). Ozone has been utilized in European countries for a long time (Guzel-Seydim *et al.*, 2004) and though

in the US ozone application in the food industry has not been widely used. However, the United States Food and Drug Administration granted generally recognized as safe (GRAS) status for use of ozone in bottled water in 1982. Ozone has also been declared as Generally Recognized as Safe (GRAS) for use in food processing by the US Food and Drug Administration (FDA) in 1997 (Graham, 1997). Furthermore, now ozone is also recognized and allowed as an antimicrobial food additive by the US Food and Drug Administration (FDA) in 2001.

Ozone has high potential applications in the food industry (Khadre *et al.*, 2001; Kim *et al.*, 1999). Food preservation through ozone is a non-thermal processing technology which helps in enhancing food safety without compromising quality and desirability of food products (Guzel-Seydim *et al.*, 2004). Ozone acts as a potential oxidizing agent and helps in eliminating pathogens due to its antimicrobial properties without leaving any residue in the treated food. Aqueous ozone may have a good potential for short-time surface treatment (decontamination) of fruit and vegetables and as a disinfectant for process water in food producing plants (Muthukumarappan *et al.*, 2000; Khadre *et al.*, 2001; Garcia *et al.*, 2003). Relatively low concentrations of ozone and short contact time are sufficient to inactivate bacteria, molds, yeasts, parasites and viruses (Kim *et al.*, 1999). There are other numerous application areas of ozone in the industry such as 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. Notably, when ozone is applied to food, it leaves no residues since it decomposes quickly (Guzel-Seydim *et al.*, 2004).

Ozone is formed naturally in the stratosphere in small amounts (0.05mg/litre) by the action of UV irradiation on oxygen. A small amount of ozone is also formed in the troposphere as a by-product of photochemical reactions between hydrocarbons, oxygen and nitrogen that are released from automobile exhausts, industries, forests and volcanic action. However, the gas produced is very unstable and decomposes quickly in the air (Khadre *et al.*, 2001). In order to generate ozone, a diatomic oxygen molecule must first be split. The resulting free radical oxygen is thereby free to react with other diatomic oxygen to form the triatomic ozone molecule. However, in order to break the O–O bond, a great deal of energy is required (Bocci, 2006; Gonçalves, 2009).

When used in industry, ozone is usually generated at the point of application and in closed systems (Gonçalves, 2009). Ultraviolet radiation (188 nm wave length) and corona discharge methods can be

used to initiate free radical oxygen formation and thereby generate ozone. In order to generate commercial levels of ozone, the corona discharge method is usually used (Duguet, 2004; Guzel-Seydim *et al.*, 2004; Bocci, 2006; Gonçalves, 2009).

3. Corona Discharge Method

There are two electrodes in corona discharge, one of which is the high tension electrode and the other is the low tension electrode (ground electrode). These are separated by a ceramic dielectric medium and narrow discharge gap is provided (Fig 1) (Goncalves, 2009). When a high voltage alternating current is applied across a discharge gap in the presence of oxygen, it excites oxygen electrons and thus induces splitting of oxygen molecules. Atoms from split oxygen combine with other oxygen molecules to form ozone. Ozone production varies depending on voltage, current frequency, dielectric material property and thickness, discharge gap and absolute pressure within the discharge gap (Khadre *et al.*, 2001). If air is passed through the generator as a feed gas, a 1-4% of ozone can be produced. However, use of pure oxygen allows yields to reach 6 to 14% ozone. Consequently, ozone concentration cannot be increased beyond the point that the rates of formation and destruction are equal. Ozone gas cannot be stored since ozone spontaneously degrades back to oxygen (Guzel-Seydim *et al.*, 2004; Gonçalves, 2009). Dried gas is used to minimize the corrosion of metal surfaces due to nitric acid deposition produced from wet gas inside the generator (Khadre *et al.*, 2001).

Advantages of Corona Discharge

- High ozone concentrations
- Best for water applications
- Fast organic (odour) removal
- Equipment can last for years without maintenance.

4. Ultra-Violet Lamp

The method is based on conversion of oxygen on ozone molecules by lamp of ultraviolet light (wavelength of 188 nm, Fig 2). Nevertheless, the ozone production is of low intensity. At low temperatures, the process of ozone ventilation is made with greater facility. The ozone production takes place generally by the ventilation of electrical discharges of high voltage in the air or pure oxygen (Guzel-Seydim *et al.*, 2004; Gonçalves, 2009). This radiation affects a common oxygen molecule that is found in atmosphere which produces the split of the molecule and separation of free oxygen atom. These atoms collide with other

oxygen molecules, forming therefore ozone molecules (Duguet, 2004; Chawla *et al.*, 2007; Gonçalves, 2009).

- The energy absorbed by an oxygen molecule break it in two oxygen atoms.
$$\text{O}_2 + h\nu \rightarrow \text{O} + \text{O}$$
- Each one of these atoms is joined to an oxygen molecule to give another one of ozone.
$$\text{O} + \text{O}_2 \rightarrow \text{O}_3$$
- Finally, the ozone molecule is destroyed again absorbing more ultraviolet radiation.
$$\text{O}_3 + h\nu \rightarrow \text{O} + \text{O}_2$$
- Ultraviolet energy is absorbed in a closed cycle of formation and destruction of the ozone.
- The ozone formed, after certain period of time, is degraded spontaneously in oxygen (Gonçalves, 2009).

Advantages of UV Light

- Simple construction
- Lower cost than corona discharge
- Output hardly affected by humidity
- Less by-products vs. corona discharge (Gonçalves, 2009).

In addition, ozone can be produced by chemical, thermal, chemo-nuclear and electrolytic methods. A new approach in producing ozone has been implemented. This is an electrochemical procedure in which water is split into hydrogen and oxygen atoms by electrolysis. The hydrogen molecules are separated from the gas and water mixture and the oxygen atoms combines to form ozone and diatomic oxygen. This procedure is shown to produce ozone at concentrations that are three to four times higher (10-18%) than those attainable by corona discharge (Khadre *et al.*, 2001).

5. Effect of Ozone on Microorganisms

Ozone destroys microorganisms by the progressive oxidation of vital cellular components. The bacterial cell surface has been suggested as the primary target of ozonation. Two major mechanisms have been identified in ozone destruction of the target organisms: first mechanism is that ozone oxidizes sulphhydryl groups and amino acids of enzymes, peptides and proteins to shorter peptides. The second mechanism is that ozone oxidizes polyunsaturated fatty acids to acid peroxides. Ozone degradation of the cell envelope unsaturated lipids results in cell disruption and subsequent leakage of cellular contents. Double bonds of unsaturated lipids are particularly vulnerable to ozone attack (Kim *et al.*, 1999). Ozone causes-

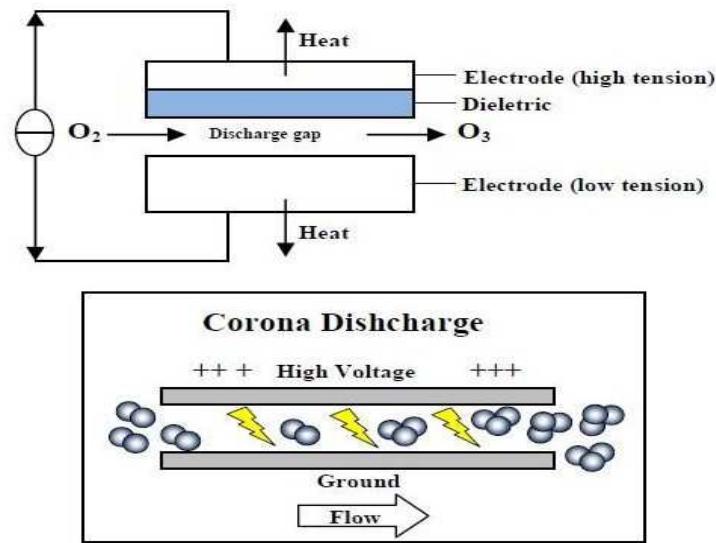


Fig 1: Scheme of Corona discharge method: Oxygen is forced between high voltage plates to simulate corona discharge. The oxygen is broken apart and recombines into ozone (adapted from Gonçalves, 2009)

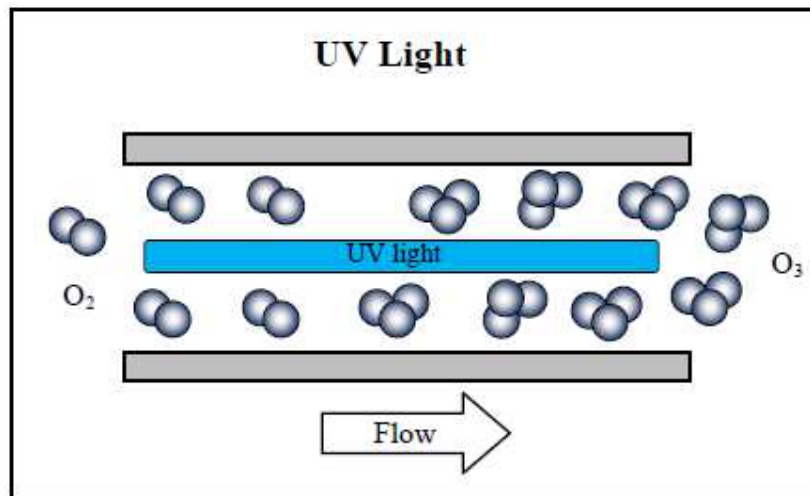


Fig 2: Ultra-violet lamp method: Oxygen turns into ozone after it is hit with UV light from a UV generating bulb (adapted from Gonçalves, 2009).

Wide-spread oxidation of internal cellular proteins causing rapid cell death. Cellular death can also occur due to the potent destruction and damage of nucleic acids. Thymine is more sensitive to ozone than cytosine or uracil. Ozone also destroys viral RNA and alters polypeptide chains in viral protein coats (Kim *et al.*, 1999; Guzel-Seydim *et al.*, 2004).

5.1 Inactivation Spectrum

Ozone is known to be effective sanitizer against all forms of microorganisms even at low concentration and at room temperature (Clark, 2004). Kim *et al.* (1999) stated that the rate of inactivation of the microorganisms is greater in ozone demand-free systems than when the medium contains oxidizable organic substances. Furthermore, susceptibility of the microorganisms to ozone also varies with the physiological state of the culture, pH of the medium,

temperature, humidity and presence of additives (such as salt, sugars, surfactants, etc.).

The influence of aqueous ozone concentrations (0.3 and 2.0 ppm), blanching (50-60°C) and three treatments combinations of microorganism/food: *Listeria innocua*/red bell peppers (artificially inoculated), total mesophiles/strawberries, and total coliforms/watercress were studied by Alexandre *et al.* (2011). In relation to ozone treatments, the highest microbial reductions were obtained for the highest concentration with the highest treatment time (3 min). Under those conditions, *L. innocua*/peppers, total mesophiles/strawberries and total coliforms/watercress were reduced respectively 2.8 ± 0.5 , 2.3 ± 0.4 and 1.7 ± 0.4 log-cycles. However, a substantial portion of the microbial populations were reduced by water washing alone, and the presence of ozone generally added an additional reduction of 0.5-1.0 log-cycles.

Restaino *et al.* (1995) showed that gram-negative bacteria were substantially more sensitive to ozone in pure water than were the gram-positive ones. Khadre *et al.* (2001) also reported that gram positive bacteria are more resistant to ozone than are the gram negative ones. Lee and Deniniger (2000) observed the dominance of gram positive bacteria among the surviving microorganisms in ozonated drinking water. Kim *et al.* (1999) showed that when compared to vegetative cells, bacterial spores have greater resistance to ozone. Also, when ozone was combined with other deleterious factors, greater inactivation rates of ozone were observed. The spore coat forms as a protective barrier against ozone. Acidic pH enhances the lethality of ozone against bacterial spores. A combined treatment of ozone and UV radiation reduces the contact time required for the inactivation of bacteria. Khadre and Yousef (2001) measured ozone efficacy against spores of 8 *Bacillus* spp. The organism *B. stearothermophilus*, which is known for high resistance to heat, also possessed the highest resistance to ozone among the species tested.

A limited number of studies on inactivation of viruses with ozone have been published. However, ozone is an effective virucidal agent (Kim *et al.*, 1999). Viruses are shown to be comparable to bacteria in sensitivity to ozone. Studies have reported that bacteriophages are the least resistant to ozone, followed by polioviruses, whereas human rotavirus was the most resistant to the sanitizer (Khadre *et al.*, 2001). Studies showed that the protozoan *Cryptosporidium parvum* (an intestinal parasite which causes gastroenteric disease) is inactivated to >90% when exposed to ozone in 1mg/litre of ozone in ozone free water (Kim *et al.*, 1999). Ozone is also an excellent fungicidal agent. Kim *et al.* (1999) reviewed that yeast is less resistant to

ozone when compared to mould. However, yeast varies in sensitivity to ozone. The antimicrobial effect of ozone on yeast increases with increased temperature, RH and treatment time.

6. Application of Ozone in the Food Industry

Today, the use of ozone is steadily replacing conventional sanitation techniques such as chlorine, steam or hot water. It's gaining momentum in the food processing industry as the safest, most cost-effective and chemical-free way of dealing with food safety management (Vaz-Velho *et al.*, 2006; Gonçalves, 2009).

Ozone has been shown to deactivate a large number of organisms, including bacteria, fungi, yeast, parasites and viruses, and can also oxidize natural organic compounds as well as synthetic substances, such as detergents, herbicides and composite pesticides (Graham, 1997; Guzel-Seydim *et al.*, 2004; Gonçalves, 2009). Ozone has been used in the food processing industry, both as gaseous ozone and dissolved in water to reduce bacteria on a wide range of food products and contact surfaces (Nash, 2002; Kim *et al.*, 1999; Guzel-Seydim *et al.*, 2004; Chawla *et al.*, 2007; Gonçalves, 2009).

7. Ozone as a Preservative Tool for Food Safety and Hygiene in the Food Industry

Ozone is one of the most powerful antimicrobial substances (natural sanitizing and disinfecting agents) in the world destroying up to 99.9% of pesticides and microorganisms commonly found on food due to its potential oxidizing capacity. Any pathogen or contaminant that can be disinfected, altered or removed via an oxidation process will be affected by ozone. It is the strongest of all molecules available for disinfection in water treatment and is second only to elemental fluorine in oxidizing power (Duguet, 2004; Gonçalves, 2009).

7.1 Fruits and Vegetables

Microorganisms are natural contaminants of fresh produce and minimally processed fresh-cut products and contamination arises from a number of sources, including postharvest handling and processing (Beuchat, 1996). It has been observed that greater than 90% reduction of total bacterial counts upon treatment of Chinese cabbages with ozonated water (2.3 mg/L) for 60 minutes. Treatment of wash water used in processing of carrots has been reported to provide 3 log reductions of bacteria. The fresh produce typically contains a complex mix of bacteria, fungi and yeast,

whose population and kinds are highly variable (Zagory, 1999; Ponce *et al.*, 2003). To prolong the shelf life of the fresh fruits and vegetables; the growth of the microbial population must be controlled (Ponce *et al.*, 2003). Chlorine is the primary sanitising agent used in fruits and vegetables washing (Reina *et al.*, 1995; Zhang and Farber, 1996; Ponce *et al.*, 2003) but it produces residual by-product such as trihalomethanes which are potential carcinogens (Fawell, 2000). Ozone is shown to be a good alternative sanitizer for fresh fruits and vegetables (Han *et al.*, 2002).

Ozone processing within the food industry has been carried out for fresh fruits and vegetables either by gaseous treatment or washing with ozonated water (Karaca and Velioglu, 2007). Two types of washing systems: spray and fume can be used to reduce microbial counts on the surface of produce (Hampson and Fiori, 1997).

Several researchers have shown that treatment with ozone appears to have a beneficial effect in extending the storage life of fresh commodities such as broccoli, cucumber, apples, grapes, oranges, pears, raspberries and strawberries by reducing microbial populations and by oxidation of ethylene (Beuchat *et al.*, 1998; Kim *et al.*, 1999; Skog and Chu, 2001). The use of ozonated water has been applied to fresh-cut vegetables for sanitation purposes reducing microbial populations and extending the shelf-life of some of these products (Beltran *et al.*, 2005a-b).

Selma *et al.* (2008) showed that gaseous ozone treatment of 5,000 and 20,000 ppm for 30 min reduced total coliforms, *Pseudomonas fluorescens*, yeast and lactic acid bacteria recovery from fresh-cut cantaloupe. A dose of 600,000 ppm/min achieved maximal log CFU/melon-cube reduction, under the test conditions. Finally, fresh-cut cantaloupe treated with gaseous ozone, maintained an acceptable visual quality, aroma and firmness during 7-day storage at 5°C. Silveira *et al.* (2007) reported a similar sanitizer effect between 150 ppm NaOCl and O₃ dips (0.4 ppm, 3 min) on FC 'Galia' melon. Both treatments reduced microbial load by 1 log CFU g⁻¹ compared to water alone and extended shelf-life to 10 days at 5°C. Tzortzakis *et al.* (2007) had inoculated tomatoes, strawberries, table grapes and plums with *Botrytis cinerea* (grey mould), transferred to chilled storage (13 °C) and then exposed them to 'clean air' or low-level ozone-enrichment (0.1 μmol mol⁻¹). Ozone-enrichment resulted in a substantial decline in spore production as well as visible lesion development in all treated fruit. Exposure-response studies performed specifically on tomato fruit (exposed to concentrations ranging between 0.005 and 5.0 μmol mol⁻¹ ozone).

There were different findings reported in the literature on the efficacy of ozone on lettuce. Olmez and Akbas (2009) carried out an investigation to optimize the ozone treatment of fresh cut green lettuce leaves. Results revealed that the application of 2 ppm ozonated water treatment for 2 min was found to be the optimum processing conditions for ozone disinfection of green leaf lettuce, in terms of reducing the microbial load and maintaining the sensory quality during cold storage. These authors used ozonated water to wash shredded lettuce. They injected 1.3 mM of ozone at a flow rate of 0.5 L/min into a water/ lettuce mixture (1:20, w/w) with highspeed stirring or before stomaching for 3 min and obtained about 2 log CFUg⁻¹ reductions in total plate counts. Rico *et al.* (2007) found that ozonated water (1mg L⁻¹ at 18–20°C) with lettuce reduced both enzyme activity and enzymatic browning. Zhang *et al.* (2005) had carried out an investigation in which fresh-cut celery was dipped with ozonated water and evaluated for changes of microbiological population and physiological quality during storage at 4°C. Treatment with ozonated water is showed to be effective to reduce the population of microorganisms and retard physiological metabolism, thus assuring microbiological safety, and nutrition value of fresh-cut celery. Results also revealed that the best preservation effect appeared to be the treatment of 0.18 ppm of ozonated water concentration, with which the microbial population was able to be lowered and nutritional and sensory quality of fresh-cut celery was maintained good for 9 days of storage at 4°C.

Beltran *et al.* (2005a) found no evidence of browning in FC potatoes dipped in O₃ water (20 mgL⁻¹ for 1 min) or in O₃ plus (300 mg L⁻¹) and stored up to 14 days under partial vacuum at 4 °C. These treatments maintained initial texture and aroma. However, the ozonated water alone was not effective in reducing total microbial populations. The combination of O₃ + Tsunami was the most effective treatment to control microbial growth achieving 3.3, 3.0 and 1.2 log-reductions for Lactic acid forming bacteria (LAB), coliforms and anaerobic bacteria, respectively. Barth *et al.* (1995) reported that ozone at 0.1 to 0.3 ppm in atmosphere during blackberry storage suppressed fungal development for 12 days at 2°C and did not cause observable injury or defects. A study by Sarig *et al.* (1996) showed that grapes exposed for 20 min to ozone (8 mg/l) had considerably reduced the counts of bacteria, fungi and yeast. Fungal decay of grapes following cold storage was reduced and shelf-life was increased on ozone treatment. Onions have been treated with ozone during storage. Mould and bacterial counts were greatly decreased without any change in chemical composition and sensory quality (Song *et al.*, 2000).

7.2 Dried Fruits

Najafi and Khodaparast (2009) showed that when ozone was applied in gas form at three concentrations (1, 3, and 5 ppm) for four different periods (15, 30, 45 and 60 min) on Iranian date fruit, there is a reduction in the total count of mesophilic microorganisms, coliforms, *S. aureus* and yeast/mould. The results also suggested that a minimum of one hour ozone treatment at 5 ppm could be successfully used for reducing the coliform and *S. aureus* of date fruits but longer exposure times are required for elimination of the total mesophilic bacteria as well as yeast/mould counts which were statistically lower than those of untreated control samples.

Zorlugenc *et al.* (2008) carried out a research on the effectiveness of gaseous ozone and ozonated water on microbial flora and degradation of aflatoxin B₁ in dried figs. The study showed gaseous ozone was more effective than ozonated water for reduction of aflatoxin B₁.

7.3 Liquid Foods

Typically, ozone processing within the food industry has been carried out on solid foods by either gaseous treatment or washing with ozonated water. However with the FDA approval of ozone as a direct additive to food, the potential of ozonation in liquid food applications has started to be exploited. A number of commercial fruit juice processors in the USA have started to employ ozone to meet the recent FDA mandatory 5 log reduction of the most resistant pathogens in their finished products (Cullen *et al.*, 2009). Effect of ozone on target microbial population, quality and nutritional parameters of fruit juices are presented in Table 2. While ozonation of liquid foods is still in its infancy, it has been reported for processing of various fruit juices including; apple cider (Choi and Nielsen, 2005; Steenstrup and Floros, 2004; Williams *et al.*, 2005) and orange juice (Angelino *et al.*, 2003; Tiwari *et al.*, 2008a).

Ozonation of liquid foods is mainly carried out in bubble columns. Bubble columns are utilised as multiphase contactors and reactors in various food, chemical, petrochemical, biochemical and metallurgical industries (Degaleesan *et al.*, 2001; Cullen *et al.*, 2009). A typical bubble column reactor is a cylindrical vessel with a gas diffuser to spurge ozone in a gaseous state into either a liquid phase or liquid-solid dispersion (Fig 3). Many empirical correlations have been proposed to estimate design parameters for a bubble column based on the physical and chemical properties of material under investigation including experimental conditions. In liquid treatment

applications, ozonation is limited by the mass transfer process governing the overall performance of ozone contactors (Zhou *et al.*, 1994; Gamal El-Din and Smith, 2002; Cullen *et al.*, 2009). The design parameters for a bubble column are: gas-liquid specific interfacial area, individual mass transfer coefficient, flow behaviour, bubble size and distribution, and coalescence of bubbles (Zhao *et al.*, 2004; Cullen *et al.*, 2009). As ozone gas solubility and diffusivity within liquids is much lower than that in the gas phase, the gas diffusion through the liquid film becomes the rate-limiting step of the mass transfer process (Cullen *et al.*, 2009). When gaseous ozone is spurge into a liquid phase, agitation occur inducing turbulent shear stresses. This causes the liquid film to become thinner. Consequently higher rates of diffusion through the liquid film occur, resulting in an increased local mass transfer coefficient (k_L). In a bubble column ozone gas interacts with liquid food, where ozone is consumed followed by a chemical reaction involving oxidation. The overall reaction rate is governed by two steps (1) the mass transfer from the gas phase to the liquid phase and (2) the chemical reaction in the liquid phase (Benbelkacem and Debellefontaine, 2003).

7.4 Spices

Ozone has been used experimentally as a substitute for ethylene oxide for the decontamination of whole and ground black peppercorns (Zhao and Cranston, 1995). Ozone treatment of ground black pepper resulted in slight oxidation of volatile oil constituents but ozone had no significant effect on the volatile oils of whole peppercorns. Because ozonation successfully reduced microbial loads and did not cause significant oxidation of the volatile oils in whole black peppercorns, this method was recommended for industrial treatment of the spice (Zhao and Cranston, 1995). Inan *et al.* (2007) had carried out an investigation to study the effect of ozone on the detoxification of aflatoxin B₁ in red pepper. Flaked and chopped samples were subjected to ozonation at various ozone concentrations (16, 33, 66 mg/l) and exposure times (7.5, 15, 30, 60 min). The results indicated that the reductions in the content of aflatoxin B₁ in flaked and chopped red peppers were 80% and 93% after exposures to 33 mg/l ozone and 66 mg/l ozone for 60 min, respectively.

7.5 Meat and Poultry

Jaksch *et al.* (2004) studied the effect of ozone treatment on the microbial contamination of pork. Commercial samples of pork meat were treated with ozone. In the study, the technique of Proton-Transfer-Reaction Mass Spectrometry (PTR-MS) was adopted to

Table 2: Lists of recent studies on ozone application in different fruit juices

Fruit juice	Phase or form	Target microbial population	Quality and nutritional attributes	Reference
Apple cider	Ozone gas (pumped into juice)	<i>Escherichia coli</i> 157:H7 (0.9 LR); <i>Salmonella</i> (1.0LR)		Williams <i>et al.</i> (2005)
Orange juice	Ozone gas (pumped into juice)	<i>Escherichia coli</i> 157:H7 (0.4 LR); <i>Salmonella</i> (1.8 LR)		Williams <i>et al.</i> (2005)
	Ozone gas (pumped into juice)	Yeast (<i>S. cerevisiae</i>) (Y)	Ascorbic acid (↓), colour (×)	Angelino <i>et al.</i> (2003)
	Ozone gas (bubble column reactor)		Colour (↑), NEB (~), cloud value (~), pH(~), TA (~), AA (↓)	Tiwari <i>et al.</i> (2008b)
Blackberry juice	Ozone gas (bubble column reactor)		Colour (↓), anthocyanins (↓)	Tiwari <i>et al.</i> (2009)
Strawberry juice	Ozone gas (bubble column reactor)		Colour (↓), pH(~), TA (~), AA (↓), anthocyanins (↓)	Tiwari <i>et al.</i> (2009a)
Grapes Juice	Ozone gas (bubble column reactor)		Colour (↓), anthocyanin (↓)	Tiwari <i>et al.</i> (2009b)
Apple cider	Ozone gas (pumped into juice)	Moulds (↓); yeast (↓)	Sediments (↑), colour (×)	Choi and Nielsen (2005)
Apple cider	Ozone gas (pumped into juice)	<i>Escherichia coli</i> O157:H7 (5.0 LR)		Steenstrup and Floros (2004)

APC, aerobic plate count; AA, ascorbic acid; PPO, polyphenol oxidase; (×), significant difference; (↑), increases; (↓), decreases; (~), no change; LR, log reduction: Source: Cullen *et al.* (2009a)

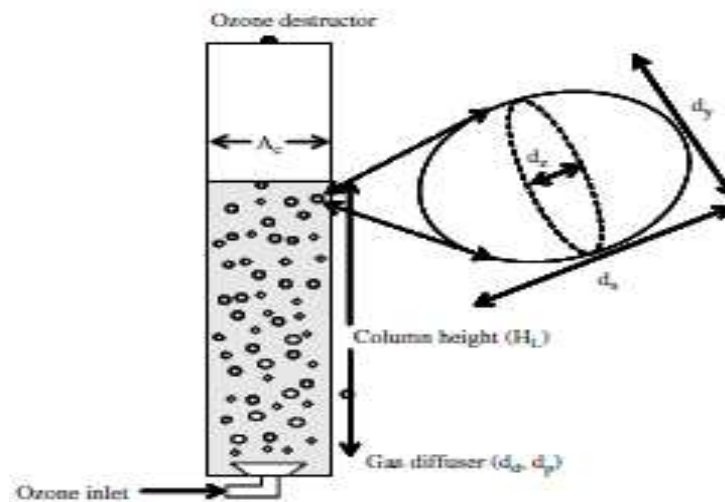


Fig 3: Bubble column reactor (adapted from Cullen *et al.*, 2009a)

study volatile emissions with the signal detected at mass 63 (assumed to be a measure for dimethylsulphide) being used as a diagnostic of bacterial activity. Such a signal was found to strongly increase with time for an untreated meat sample whereas ozone-treated meat samples showed much reduced emissions-suggesting that the microbial activity had been greatly suppressed by ozone treatment, thus increased in shelf-life of the meat.

A study was carried out by Castillo *et al.* (2003) in which to measure the effectiveness of an aqueous ozone treatment in reducing *Escherichia coli* O157:H7 and *Salmonella* serotype Typhimurium on beef carcass surfaces. The test samples were exposed to a water wash followed by a sanitizing ozone treatment. Under the conditions of this study, results revealed that the aqueous ozone treatment applied to test samples did not show any significant improvement in reducing pathogens on beef carcass surfaces when compared to the control samples exposed to a water wash alone.

The effects of beef trimming decontamination with ozone and chlorine dioxide on ground beef microbial, colour and odour characteristics were studied by Stivarius *et al.* (2002). Beef trimmings were inoculated with *Escherichia coli* and *Salmonella typhimurium*, then treated with either 1% ozonated water for 7 min, 15 min or with 200 ppm chlorine dioxide and compared with a control. The results revealed that all the treatments were found effective for reducing microbial pathogens with minimal effects on colour or odour characteristics. However, the authors suggested that additional work might focus on concentration and exposure times necessary to optimize antimicrobial properties.

Al-Haddad *et al.* (2005) had inoculated chilled breasts of chicken with *Salmonella infantis* or *Pseudomonas aeruginosa* and then subjected them to one of the following treatments: (i) exposure to gaseous ozone (>2000 ppm for up to 30 min); (ii) storage under 70% CO₂:30% N₂; and (iii) exposure to gaseous ozone (>2000 ppm for 15 min) followed by storage under 70% CO₂:30% N₂; all storage at 7 °C. Gaseous ozone reduced the counts of *S. infantis* by 97% and by 95%, but indigenous coliforms were unaffected. Under the modified atmosphere, the cell count of *S. infantis* was reduced by 72% following initial exposure and then stabilised, coliforms grew, but *P. aeruginosa* behaved like *S. infantis* -initial reduction (58%) followed by stability. Exposure to gaseous ozone followed by gas packaging allowed survival of *S. infantis*, *Ps. aeruginosa* and coliforms over 9 days at 7°C, but there was no evidence of any sensory deterioration. It is proposed that the latter treatment could, in a modified form perhaps, be used to

reduce the contamination of chicken carcasses with salmonellae and improve their shelf-life.

7.6 Sea Foods

Ozone application for fresh fish helps suppress the characteristics smell which sometimes can be disagreeable, hence giving a healthful aspect to the fish (Gonçalves, 2009). Ozone pre-treatment (6 ppm) of tilapia helps to increase shelf life of the product by 12 days. The combination of ozone pre-treatment with storage at 0°C appears to be a feasible means of prolonging the storage life of fish (Nash, 2002; Gelman *et al.*, 2005). Ozonized water was used for dipping and washing fish or fish fillets showed an effective reduction of microbiological flora and had no effect on the product (Tapp and Sopher, 2002; Gelman *et al.*, 2005, Gonçalves, 2009). The catfish showed highly statistically significant reductions in plate counts when live fish and fillets were washed in ozonated water (Tapp and Sopher, 2002; Campos *et al.*, 2006).

Ozonated water technology can be successfully used as a germicidal agent in seafood processing to extend the shelf life and quality of wild shrimp in a time when efforts are being made to eliminate the use of commonly used chlorine due to its ability to form potential carcinogens on reacting with organic matter (Graham, 1997; Chawla *et al.*, 2007). Chawla *et al.* (2007) indicated that the shelf life of shrimp stored in ice based on bacterial loads was slightly extended by soaking in 3 ppm O₃ for 60 seconds. It also reported that though the bio-amine production was not found to be reduced by the treatment, but consumer sensory scores did indicate higher acceptability of ozone treated shrimp samples at the end of the shelf life study.

Also, it was found that soaking peeled shrimp meat in ozonated water is more effective than spraying shrimp with ozonated water, and the higher ozone concentrations and longer treatment times were more effective for reducing levels of spoilage bacteria levels on the shrimp. The application of ozonated water did not increase lipid oxidation in the shrimp immediately after treatment (Chawla *et al.*, 2007).

Manousarides *et al.* (2005) studied the effect of ozone on microbial, chemical and sensory attributes of shucked mussels for a period of 12 days. The study revealed that the ozonation affected the bacterial population namely, aerobic plate count 0.7-2.1 log cycle reduction, *Pseudomonas* spp. 0.5-1.1 log cycle reduction, H₂S producing bacteria 1.1-2.5 log cycle reduction and *Brochothrix thermospecta* 0.3-1.4 log cycle reduction. The effect of ozone was more pronounced at longer exposure time. Chemical indicators such as Trimethylamine remain low throughout the storage period and total volatile basic

nitrogen remains low till six days of storage. The shelf life of the treated mussels was 12 days as compared to the control samples.

7.7 Grains and Their Products

Ozone is an effective fumigant for insect killing, mycotoxin destruction and microbial inactivation which has a minimal or no effect on grain quality. Studies have demonstrated that ozone which is a natural agent, may offer unique advantages for grain processing along with addressing growing concerns over the use of harmful pesticides (Tiwari *et al.*, 2010).

Sousa *et al.* (2008) studied the effect of ozone toxicity to phosphine-resistant and susceptible populations of *Tribolium castaneum* (Herbst), *Rhyzopertha dominica* (F.) and *Oryzaephilus surinamensis* (L.) collected from different regions of Brazil. As none of the populations showed resistance to ozone, regardless of their susceptibility to phosphine, ozone is a potential alternative for phosphine resistance management in the insect species evaluated in this study.

Mendez *et al.* (2003) investigated the flow characteristics of ozone through less porous grains and the effects of long exposure to a high ozone concentration on the grain quality for end-users of the grain. The study indicated that treatment of grains with 50 ppm ozone for 30 days had no detrimental effect on the popping volume of popcorn, on the fatty acid and amino acid composition of soybean, wheat, and maize. There was no effect on the milling characteristics of wheat and maize, baking characteristics of wheat as well as on the stickiness of rice.

Ibanoglu (2001) reported that ozonated water up to a concentration of 11.5 mg/lit can be successfully used in tempering of soft and hard wheat. The use of ozonated water did not change the chemical and physical properties of the parent flour regarding the baking and milling parameters. Furthermore, a statistically significant reduction in the total bacteria and yeast/ mould populations in the wheat kernels tempered using ozonated water.

8. Use of Ozone for Sanitization of Plant Equipments

Canut and Pascual (2007) showed that ozone might play an advantageous role in cleaning in place (CIP) operations, in various sectors of the food industry, as an alternative to other sanitizers with several potential environmental advantages. Guillen *et al.* (2010) tested the efficacy of ozone in a CIP system of a wine industry. In this study, a hose that transported wine was submitted to the following treatments: ozonated water at 28±1 °C; hot water; peracetic acid

and a caustic soda solution with peracetic acid. The results indicate that the use of ozonated water is more effective than the isolated use of peracetic acid and the combined use of soda and peracetic acid.

Pascual *et al.* (2007) showed that adopting ozone in cleaning and disinfection processes can bring various advantages over commonly employed disinfectants. Ozone breaks down quickly into oxygen without leaving undesirable residues. This is an advantage both from the point of view of food safety and to improve the quality of wastewaters by avoiding the presence of harmful chlorine compounds. Replacing chemical products with ozone also lowers the concentration of salts and, therefore, the electrical conductivity of discharges. The use of ozone can also save water in comparison to other biocides, as it is faster-acting. Additionally, since it does not leave residues it does not require a final rinse to remove any residual disinfectant that might remain in the treated medium. Another advantage, provided adequate microbiological controls are implemented, is that the ozonated water that has been used for disinfection can potentially be re-used for the initial cleaning stages, either directly or after re-ozonation to attain the required quality. Ozone use also provides energy savings as it is normally used at low temperatures. Finally, as it is generated ‘‘on the spot’’, ozone removes the need to store hazardous substances which could give rise to accidents that endanger human and environmental health and safety.

9. Food Packaging Materials

Another area of application of ozone is in sterilization of packaging materials. Five-log reduction was observed in the bacterial count of plastic films treated with ozonated water (Khadre and Yousef, 1999). Ozone mainly reacts with the surface of the polymers and causes modification of the surface properties of polymers such as polarity and surface tension due to the formation of oxygen containing functional groups and degradation of the polymer chains. Plastic films with low surface tension have poor adhesion properties. However, ozone treatment significantly increased the surface tension and hydrophilicity of polymers such as PE, PP and PET, and improved their adhesion properties (Mathieson and Bradley, 1996; Ozen and Floros, 2001). Rate of oxidation and changes in the properties of polymers as a result of exposure to ozone depend on the chemical structure of the polymer. PS films have lower stability against ozone compared to the stability of the other plastic films and this is attributed to the aromatic ring in its structure, and this aromatic ring is designated as the site of ozone attack (Ozen and Floros, 2001).

Karaca and Smilanick (2011) studied the influence of plastic composition and ventilation area on ozone diffusion through some food packaging materials. Gaseous ozone was applied to several common plastic films with a range of ventilation areas and diffusion through them determined. The extent of ozone diffusion followed the sequence of high-density polyethylene > polypropylene > low density polyethylene, differences among them were small and not significant. Gradual but modest increases in ozone diffusion occurred as the ventilation area increased. Since ventilation area had a significant but modest influence on O₃ diffusion, packages with optimized ventilation ratios that prevent quality loss but allow adequate contact to sanitizing agent (ozone, sulfur dioxide etc.) should be selected or designed, if none of the current commercial packages are acceptable.

Khadre and Yousef (2001) showed that sterility of multi laminated packaging materials can be achieved when 1.0 X 2.0 cm- pieces of the naturally contaminated material was treated with ozone in water (5.9 µg/ml) for 1 min. Dried films of spores were eliminated from the surface of the material when exposed to 13 µg/ml of ozone. *Pseudomonas fluorescence* in bio films on the multilaminated packaging material was eliminated up to 18⁸ cfu / 12.5 cm² when repeated exposed to ozone. The results concluded that ozone is an effective sanitizer with potential application in the decontamination of packaging materials.

10. Other Applications of Ozone (or Ozonized Water)

- Air Treatment: to purify the atmospheres contaminated with volatile organic compounds and microorganisms.
- Water Treatment: to reduce in great amount the chlorine use, without form chlorinated organ compound (Rice, 1997; Goncalves, 2009).
- Medicine/dentistry: like active medicine and dental surgery, presenting and displaying viral inactivation, bactericidal and fungicide effect (Bocci, 2006; Azarpazhooh and Limeback, 2008; Goncalves, 2009).
- Industrial Processes: to reduce the use of chemical agents, as chlorine; to reduce the residual pesticides on food (Wu *et al.*, 2007; Goncalves, 2009).

Advantages

Ozone technology has several significant advantages over its chemical alternatives as suggested

by (Young and Setlow, 2004; Chawla *et al.*, 2007; Goncalves, 2009)

- It is the strongest oxidant and disinfectant available commercially for the treatment of aqueous solutions and gaseous mixtures contaminated with oxidizable pollutants and/or microorganisms.
- Ozone can be generated on-site.
- Ozone is one of the most active, readily available oxidizing agents.
- Ozone rapidly decomposes to oxygen leaving no traces.
- Reactions do not produce toxic halogenated compounds.
- Ozone acts more rapidly, and more completely than other common disinfecting agents do.
- Ozone reacts swiftly and effectively on all strains of all kinds of microorganisms.
- Although only partially soluble in water, it is sufficiently soluble and stable so that its oxidation and/or disinfection properties can be utilized to full advantage.
- As ozone does its oxidation/disinfection work, or when it auto-decomposes, the stable end-product from ozone itself is oxygen; and reacts with a large variety of organic compounds, although at varying rates.

Disadvantages

Ozone technology however has few disadvantages (Goncalves, 2009).

- High capital cost compared with other oxidation/disinfection techniques due to the fact that the ozone must be generated on-site, thus eliminating the usual savings from centrally produced chemicals.
- The currently most economical generation of ozone in commercially significant quantities (by corona discharge) is an electrically inefficient process due to the fact that more than 75% of the electrical power sent to a corona discharge generator is converted into heat and light. Therefore, the major operating cost of producing ozone is the electrical energy.
- While ozone is a potent oxidant and can reduce bacterial levels in pure culture, the use in food processing operations where bacteria exist within organic material is more difficult.
- Since ozone is the most powerful oxidizing agent available, it is also potentially the most dangerous of oxidants. This danger was

recognized in the early stages of ozone research and techniques have been developed to insure the absence of ozone accidents.

11. Future Perspective

As we can see above, ozone has found applications in various sectors in the food industry. It's potential to warrantee microbial quality of the various products as well as a potent sanitizer for plant equipments clearly predicts that this technology has a bright future and potential in the industry. However, the following conditions cannot be out looked.

- The effectiveness of ozone being influenced by many factors, such as, method of the use, temperature and pH, besides the quality of used water.
- Ozone having a negative impact on the anthocyanin and colour of fruit juices.
- And, the facts that microbial cross contamination in workplace environment remains to be a leading cause of foodborne illness.

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Therefore, better sanitation agents and enhanced sanitation regimens will be needed to minimize the spread of harmful bacteria to finished products. Thus, more research must be done to demonstrate the best concentrations and the best methods of application of ozone in the different sectors of food industry so as to obtain safe and wholesome products as well as to maintain hygiene within the industry.

12. Conclusion

There is great potential for using the reactive, antimicrobial properties of a natural environmental friendly compound such as ozone when synthesized in a controlled system for food-based applications. Although ozone technology has existed for over a hundred years, its recent acceptance fuelled by environmental and health concerns now poises this technology for future longevity and increased successful usage, whether based on water purification, water recycling, air quality improvement, product extended storage and/or equipment surface sanitation.

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