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Sanitization Potential of Ozone and Its Role in Postharvest Quality Management of Fruits and Vegetables

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Abstract
The worldwide consumption of fruits and vegetables has witnessed a surge in the recent past which has led to an increase in the frequency of foodborne illnesses associated with fresh produce. For an effective food contamination control, conventional sanitization methods have recently been under scrutiny due to the production of undesirable and harmful by-products. As such, potential alternatives are being sought by the fresh and fresh-cut industries that can effectively eliminate pathogenic and spoilage-causing microorganisms and, at the same time, leave minimal or no residues in the product. Recent developments in the ozone technology along with its globally acknowledged regulatory status have made its integration in the food processing line easier. However, nonoptimization of process parameters and variability in working conditions has led researchers to often arrive at contradictory results. This review paper is aimed to give a detailed outline of the potential of ozone in providing efficient sanitization of fresh produce and the role of technological parameters and ozonation conditions and their effect on nutritional and sensory quality of the treated produce.

Keywords Ozone · Fresh-cut produce · Sanitizer · Kinetics · Quality

Introduction

Health consciousness among people has seen a rising trend in the recent past. As such, the demand of fresh fruits and vegetables has been ever increasing. In 2013, the total worldwide production of fruits and vegetables was 656.5 and 794.2 million tonnes, respectively [35]. India is the second largest producer of fruits and vegetables in the world, where China holds the apex spot. Fruits and vegetables, besides being ready-to-eat, are full of nutritious components and are regarded as beneficial to health. Fresh-cut and minimally processed produce are among the commodities with a higher demand by salad bars, restaurants, and fast food services over the recent past. However, with this increased consumption comes an increased risk of foodborne illnesses arising, caused by consuming contaminated products [120]. Freshly harvested fruits and vegetables are very perishable and are prone to spoilage during production, transportation, and storage [61]. The kind of spoilage-causing microbes can vary with the type of produce, cultivation methods, harvest conditions, and geographical location [16]. The spoilage-causing microbes in fruits and vegetables are fungi (*Botrytis* spp., *Alternaria* spp., *Colletotrichum* spp., *Monilinia* spp., *Diplodia* spp., *Penicillium* spp., *Sclerotinia* spp., and *Rhizopus* spp.) and bacteria (*Pseudomonas* spp. and *Erwinia* spp.). However, other pathogens such as *Listeria monocytogenes*, *Escherichia coli*, *Enterococcus faecalis*, and *Salmonella typhimurium* are also given special attention [34, 110]. Microbial contamination of fresh produce is believed to cause 2–8% of all foodborne illnesses [97]. For instance, in 1991, traditionally pressed apple cider was linked with the outbreak of *E. coli* O157:H7 infections and hemolytic uremic syndrome. Similarly, 21 juice-associated outbreaks were reported in the USA from 1995 to 2005 [135]. These outbreaks have shown that foodborne pathogens can spread through improperly processed fruit juices. In the same way, the use of contaminated produce in ready-to-use salads or garnishes can spread pathogenic microorganisms. Therefore, ensuring the safety of these products has gained important attention. Sanitizing methods to reduce and eliminate spoilage-causing microbes in general and human pathogens in particular have been a hotbed of research works.

Various sanitizing methods to safeguard fresh fruits and vegetables have evolved over time. Washing with tap water has been probably the oldest sanitizing method. However, it

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does not provide an efficient sanitization and its effectiveness also depends on the quality of the water used [12]. Commercially, washing with chlorinated water has been the traditional method to reduce microbial load from both whole and minimally processed produce [42]. However, chlorine has been reported to react with organic compounds to form potentially harmful by-products such as haloacetic acids and trihalomethanes [100]. As such, proper rinsing of treated produce after chlorine sanitization becomes a critical step for ensuring permissible levels of these by-products in the food material [45]. Furthermore, its effectiveness is limited (under permitted doses) against spore-forming microbes [50], pathogenic bacteria [105], viruses [129], and protozoan cysts [72], particularly at high pH. In addition, prolonged exposure to chlorinated water can induce off-flavors and hence alter the quality of fresh and fresh-cut produce [52]. As such, law-enforcing agencies like that of the European Union legislation (e.g., EEC 2092/91) and others have imposed strict regulations restricting the use of chlorine and bromine-based sanitizers in the fresh-cut industry with the focus on completely phasing out their use [106]. As a consequence, an alternative sanitizing agent that is safe and at the same time effective against pathogenic microbes is sought out by the industry. As such, novel, green, and efficient sanitizing methods using ozone, ethanol, hydrogen peroxide, organic acids, electrolyzed water, ultraviolet radiation, ultrasound, and high-pressure processing have recently garnered a high interest [6, 36]. Alternative methods with or without the use of conventional sanitizing techniques have been encouraged to prevent cross contamination of fresh produce [85]. Ozone, both as gas and in aqueous form, is potentially the most promising alternate sanitizing agent and has been used as a water disinfectant since the late nineteenth century [47]. However, due to a strong oxidizing effect, exposure to ozone may have detrimental health effects including headaches, coughing, dry throat, shortness of breath, a heavy feeling in the chest, and fluid in the lungs. The severity of symptoms may increase with longer exposure or higher concentration of the gas. As such, precautionary measures must be taken to ensure a safe working environment in a food processing plant involving ozone sanitization [94].

Engineering and Technological Aspects of Ozonation

It becomes imperative for a food processor working with ozone to understand the technological aspects associated with using the gas as a sanitizer. The knowledge of basic properties, factors affecting the sanitizing efficiency, kinetics of degradation, equipment material compatibility, and so on is necessary to understand the mechanisms involved with ozonation and these have been covered in the following subheadings.

Properties of Ozone Gas

Ozone (O_3) is triatomic oxygen and is formed when molecular oxygen (O_2) reacts with a free radical of oxygen. It has a characteristic pungent smell [71] and, at ordinary temperature, is a blue gas when generated from dried air, but colorless when generated from high-purity oxygen. Regardless of generation source, for normally produced concentrations, including for food processing, color is not detected. Gaseous ozone has a molar mass of 47.998 g/mol, enthalpy of 142.3 kJ/mol, gas density of 2.14 g/L, boiling point approximately equal to − 112 °C, and melting point of − 192 °C. It has a higher oxidation–reduction potential (ORP = 2.07 mV) as compared to chlorine (1.36 mV) and hypochlorous acid (1.49 mV), but lower than fluorine (3.06 mV) [49, 82]. Ozone is denser (2.14 g/L) than air (1.28 g/L) at 0 °C and atmospheric pressure [90]. The solubility of ozone is almost 13 times as compared to oxygen, which decreases with increasing temperature [111]. As a gas, ozone decomposes rapidly at room temperature, but has a greater half-life in gaseous state than in dissolved state. Almost 50% of ozone destroys in 20 min at 20 °C in tap or distilled water, whereas in double distilled water, only 10% breaks down after 85 min at the same temperature [55]. Hence, ozone is a rapidly decomposing gas that disintegrates into atmospheric oxygen, thus leaving no residues of its own. On the contrary, it has been reported to reduce chemical residues in foods from insecticidal and fungicidal applications [58, 59, 93]. In a recent study conducted by de Souza et al. [29], the removal percentage of difenoconazole and linuron in carrots was found to increase with an increase in ozone concentration and the time of treatment. Ozone application was found to promote the removal of more than 80% of pesticides when the roots were exposed for 120 min at 5 and 10 mg/L.

Ozone Generation and Application Principle

In the process of ozone generation, oxygen molecules are split to make free radicals which then react with the oxygen molecule to form ozone. A great deal of energy is though required to break the bond. This energy is supplied through ultraviolet radiation (185 nm wavelength) or high energy electric field. The latter, called the corona discharge (CD) method/plasma technique, is commonly used in commercial applications [131]. Ozone is generated using the CD method by passing a dried, dust-free, oil-free, oxygen-containing gas through two special electrodes producing a high energy electric field. In the process, diatomic oxygen is cleaved and free radicals thus produced react with the diatomic oxygen to produce ozone. A schematic layout of ozone generation using the CD and UV methods is presented in Figs. 1 and 2, respectively. If air is used for ozone generation, an ozone yield of 1–3% is obtained, while a production of 16% could be obtained if high-purity oxygen is used [111]. The high energetic processes that

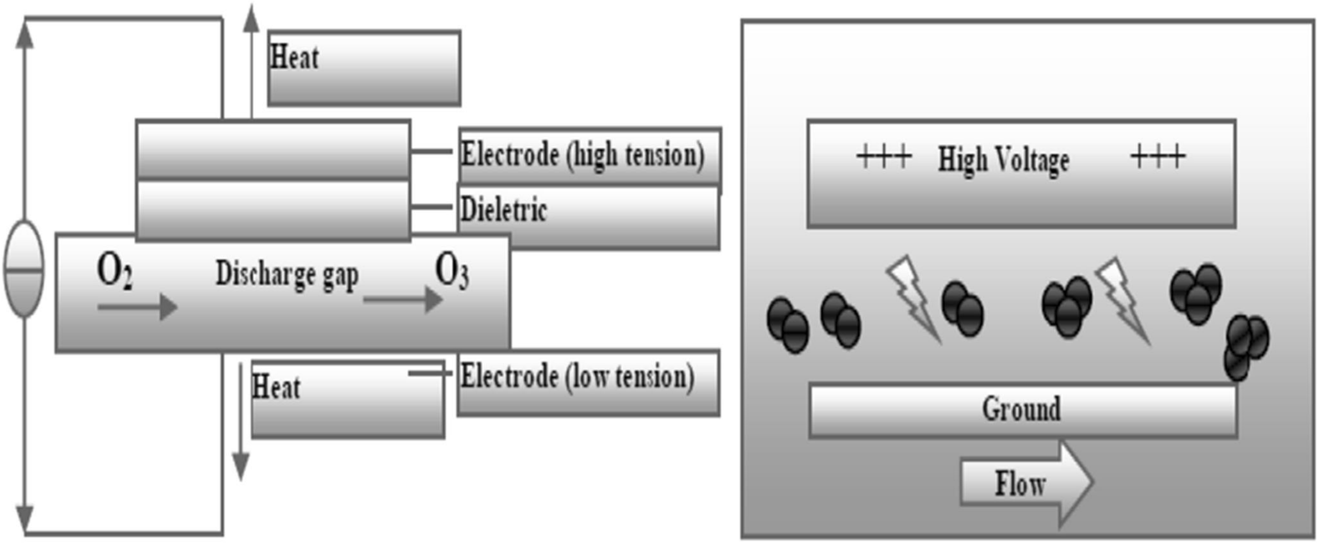

Fig. 1 Schematic diagram of Corona Discharge technology [46]

produce ozone also have the capability to destroy it. A point is reached where the rate of formation and that of degradation become equal and ozone concentration cannot be increased beyond that point (Manley and Niegowski 1967). In the process of generation of ozone, selectivity of the generation method depends on the requirement of gas output and purpose of application.

After generation of ozone gas, the next step is to apply it to the target material for disinfestation, which can be done either in gaseous or in dissolved form. In the dissolved form, the controlling variable is the partial solubility of the gas in water (Henry's law). It can be expressed mathematically as:

$$C_s = BMP_g$$

where C_s is the dissolved gas concentration in milligrams per liter, M is the phase density of gas in milligrams per liter, P_g is the partial pressure in atmospheres, and B is the Bunsen's absorption coefficient.

The solubility of ozone in water is affected by a number of physical parameters like temperature, pH, presence of readily oxidizable compounds, ionic strength, and ozone demand of the water used.

For the purpose of application, ozone gas can be made to contact with water via two methods: venturi injection and fine bubble diffusion, depending upon the specificity of the food processing operation. In the former method, water and ozone-containing gas are made to enter into the venturi through different inlets maintained at a vacuum initiated by a minimal pressure differential between outlet and inlet sides. When pressurized liquid is passed through the injector inlet, its velocity increases with a simultaneous drop in pressure, enabling for a thorough mixing of gas and water. In the latter method, pressurized ozone from ozone generator is made to expand through a porous stone or frit into the target liquid. A counter-current arrangement for gas–water mixing with water flowing downward and the gas made to flow upward is usually advised to obtain a higher contact time between gaseous ozone and water. The pressure of ozone gas should be maintained at a greater level than that exerted by the liquid at the bottom for homogeneous mixing [90]. However, by maintaining a higher pressure in the fine bubble diffuser method, it

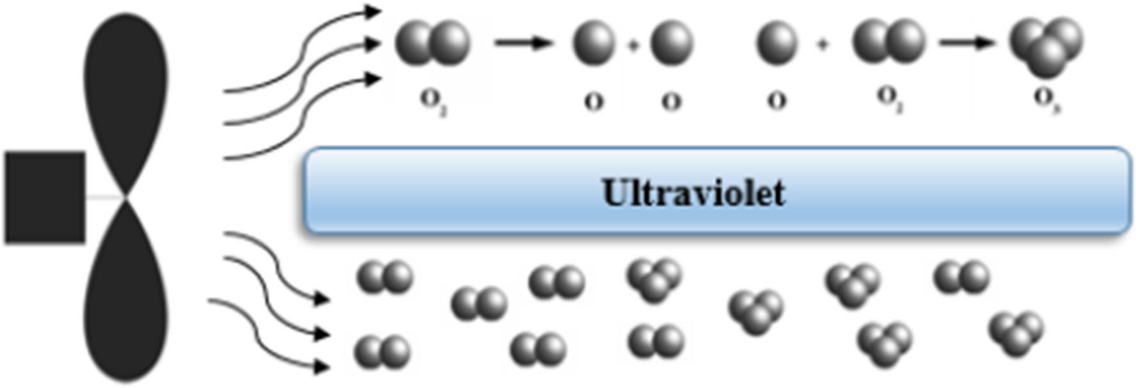

Fig. 2 Schematic diagram of ozone generation using UV

becomes less safe than venturi injection in the case of a possible leak into the working environment. In addition, for a real-time industrial approach, turbine diffusers and static agitators can be employed to achieve higher degrees of dissolution and contact [10].

Ozone Measurement and Control

In order to ensure the effectiveness of the ozone treatment, it is necessary to measure and monitor ozone levels so that the required levels for sanitization are achieved. The effectiveness of the ozone treatment process is expressed in terms of 'Ct value' (mg/min/L). It is the product of the concentration of ozone (mg/L) and the time of contact of ozone with the food product (min). Hence, a Ct value of 1 implies that 1 mg/L of ozone is applied for 1 min. A Ct value of 0.48 mg/L/min for ozone sanitization has been found to reduce the population of most microorganisms in food processing plants by 99.9% or by 3 log reductions [100]. Dissolved ozone concentrations can be conveniently measured by colorimetric test kits or by using electronic meters. In the former method, ampoules containing reagents sealed at negative gauge pressures are placed on the ozone-containing liquid. When the ampoule tip is snapped off, a sample is drawn into the ampoule, and reaction proceeds. The mix is placed in the cell holder of a photometer. Observed absorbance values are converted to milligrams per liter from calibration charts provided by the manufacturer. In the latter method, a membrane-covered amperometric sensor containing a gas-permeable membrane is stretched tightly over a gold cathode. The internal circuit is completed by a silver anode and an electrolyte solution. Once ozone enters into the sensor, it reacts to form an intermediate compound, producing a current between the anode and cathode, which is measured by the analyzer. This rate is proportional to the rate of ozone diffusing through the membrane and gives the concentration. This method can measure both, gaseous ozone as well as the dissolved gas concentration. However, this method is expensive and requires a large capital investment. In gaseous form, ozone has been successfully used as a fumigant in grain storage as well as to reduce fungus, molds, and other microbial appearances on fresh produce stored in an ozone-enriched controlled atmospheric storage. Moreover, ozone gas is known to destroy ethylene that enhances ripening of many fruits and vegetables [9]. In gaseous phase, ozone is quantized by two widely used methods in food processing applications. These are the ultraviolet absorption (UVA) method and metal oxide semiconductor (MOS) technology. The UVA method uses the absorptivity of ozone and the concentration is calculated by Beer Lambert's law. This method is mostly preferred as it gives very efficient monitoring and control up to a least concentration of 0.10 ppm ozone in a gas mixture. The results obtained are very accurate and technically more complex, but the analyzers are more expensive [126]. In contrast, ozone analyzers employing the MOS method are used where precise control over ozone concentration is less critical and have found applications as an alarming unit to detect whether ozone levels have surpassed a safe level in a food processing plant [103]. They are less expensive than UVA analyzers and are available as handheld, wall-mounted, or even in a clip-on format [126].

Components of the Ozonation System

In an ozone sanitization process, ozone gas requires to be produced onsite as it degrades quickly and cannot be stored ([24, 71, 139]). The raw material for ozone generation is atmospheric oxygen which is abundantly available and inexhaustible. However, oxygen present in the atmosphere needs to be concentrated using an oxygen concentrator to obtain higher levels of feed gas. An ozonation system in a food processing plant usually involves air treatment to concentrate oxygen, ozone generator, flow meters, concentration monitors, treatment chamber, and an ozone destructor to disintegrate unused ozone before releasing it to the atmosphere [107]. Moreover, in the case of aqueous ozone, special interface equipment like the venturi meter or diffusers are used to obtain higher degrees of homogeneous gas dissolution.

In ozonation processes, ozone is generally produced by either the photochemical (UV) or CD method, which are two main accepted procedures. There have been reports of several nonconventional methods of ozone generation in the literature like electrolysis, reaction of elemental phosphorus with water, or radiochemical reactions. However, these methods are still in initial stages of development and are not feasible for food processing operations at this time. The CD method of ozone generation is more preferred because of its advantages over the UV method as presented in Table 1. The principle of ozone generation with the CD method usually involves passing an oxygen-enriched, clean, and dry gas through a high energy electric field produced between two electrodes. One of the electrodes is used as a dielectric, current bearing and the other as a ground electrode. A high input electric field uniformly energizes the surface of the dielectric made of ceramics or scientific grade glass. The space between the electrodes is called the reaction chamber, where a high energetic field (corona) is formed that splits oxygen into very active oxygen radicals that react with molecular oxygen to produce ozone [77]. Conventionally, ozone generators employing the CD method operate at a low frequency (50–60 Hz) and a high voltage (> 20,000 V), but more recently, generators requiring a higher frequency (1000–2000 Hz) and 10,000 V have been reported to generate ozone more effectively [19]. The outlet gas flow from ozone generators is usually controlled with a manual volume control switch that varies the concentration from 0 to 100%. However, for more sophisticated generators used in food industries, an automatic

Table 1 Comparison of photochemical (UV) and corona discharge methods (CD) of ozone generation [137]

Parameter	Photochemical (UV) method	Corona discharge (CD) method
Specific energy consumption/g O_3 produced	0.515 kWh/g O_3 using 185 nm UV	0.018 kWh/g O_3 from dry air
Maximum rate of ozone generation	1.94 g/kWh	55 g/kWh
Concentration of O_3 in output feed by weight	1.8 g/m^3 0.14%	12–60 g/m^3 0.1–4.8%
Initial investment	Low	High
Operating cost (electrical energy)	High	Low
Output ozone flow	Variable	Constant
Pretreatment of ambient air	Desired but not necessary	Necessary

ON/OFF or 4–20 mA control has found real-time applications. The ozone generator can be controlled by programming the data collected from dissolved ozone monitors or sensors that detect the oxidation–reduction potential. A reading toward 20 mA would correspond to a decline in ozone concentration, while a reading toward 4 mA would be obtained as the ozone level approaches the high set point [126]. Moreover, data from these device controllers can be stored in a data logger to help the processor in maintaining records. As a virtue of advancements like these in ozone engineering, achieving higher quality standards for sanitization has become possible.

Since for most food processing applications ozone is generated by the CD method, atmospheric air needs to be pretreated to enrich the concentration of oxygen for ozone generation. Oxygen concentrators provide > 90% O_2-enriched gas feed to ozone generators in a convenient manner. These devices work on the principle of pressure swing adsorption (PSA) where an air compressor compresses the cleaned dry ambient air and transfers it to a molecular sieve bed where nitrogen and water vapors are trapped and thus resulting in an oxygen-enriched outlet stream that is fed to an ozone generator. The adsorption capacity of the sieve bed is maintained by desorption of nitrogen and moisture-loaded beads as waste in vapor form to the atmosphere, hence refreshing the seat of adsorption. As such, most oxygen concentrators employ multiple chambers for simultaneous pressurization and adsorption and depressurization and desorption, respectively ([90]). One of the most prominent knowledge gaps in using ozone as a sanitizer in the food processing industry has been the optimization of a treatment system, which provides the seat of the sanitization process. Most of the approaches tested to date have been on the laboratory scale, although several are advancing toward commercial application. Ozonation systems used in laboratories for treating fresh and fresh-cut produce with aqueous ozone involve mainly a glass or a stainless steel tank with provisions of ozone and water inlet and outlet. For example, Brodowska et al. [17] developed an apparatus for decontamination of cardamom seeds with gaseous ozone. The apparatus consisted of a cylindrical reactor made of glass inside a steel chamber in which the seeds were continuously treated with oxygen/ozone mixture and was equipped with a control system having a jolting and rotating mechanism to intensify the movement of plant material within the reactor. Similar systems can be used for the treatment of plant produce with aqueous ozone; however, the pH of water should be continuously monitored ([87]; Brodowska et al. [18]). Similarly, Ketteringham et al. [65] developed a system for ozonation of precut green peppers. The system consisted of a plastic water barrel where water was ozonated prior to application (Fig. 3). The gas was contacted with water by a diffuser placed in the bottom of the tank which dispersed the gas stream into fine bubbles that rose in the water column. Similarly, Wani et al. [136] designed an ozone fumigation system for treatment of spinach with gaseous ozone. The system consisted of a stainless steel chamber covered by a pyrex cover (Fig. 4). The ozone gas inlet to this chamber was provided at the bottom and excess gas was removed from the other side. An ozone destructor in an ozonation system is an essential part that disintegrates excess ozone to atmospheric oxygen before passing the waste air stream into the ambient environment outside the processing units. These equipments work on the catalytic principle and contain a catalyst, like MnO_2 or activated carbon with a high oxidation demand to disintegrate ozone to atmospheric oxygen and ensure safe disposal of waste air stream.

Equipment Material Compatibility for Ozonation

The application of ozone for processing fresh fruits and vegetables should not pose any harm to the materials of construction and to the food itself. The potential of different materials to withstand the effects of ozone at concentrations around 1000 ppm is shown in Table 2 [25]. The rating of the materials varies from poor to excellent depending upon the stability of the material upon exposure to ozone. Materials like HDPE, stainless steel, and glass exhibit excellent resistance to the oxidizing effect of ozone, while organic materials like natural rubber and nylon and materials like magnesium, zinc, and mild steel are readily oxidized by ozone. The difficulties in

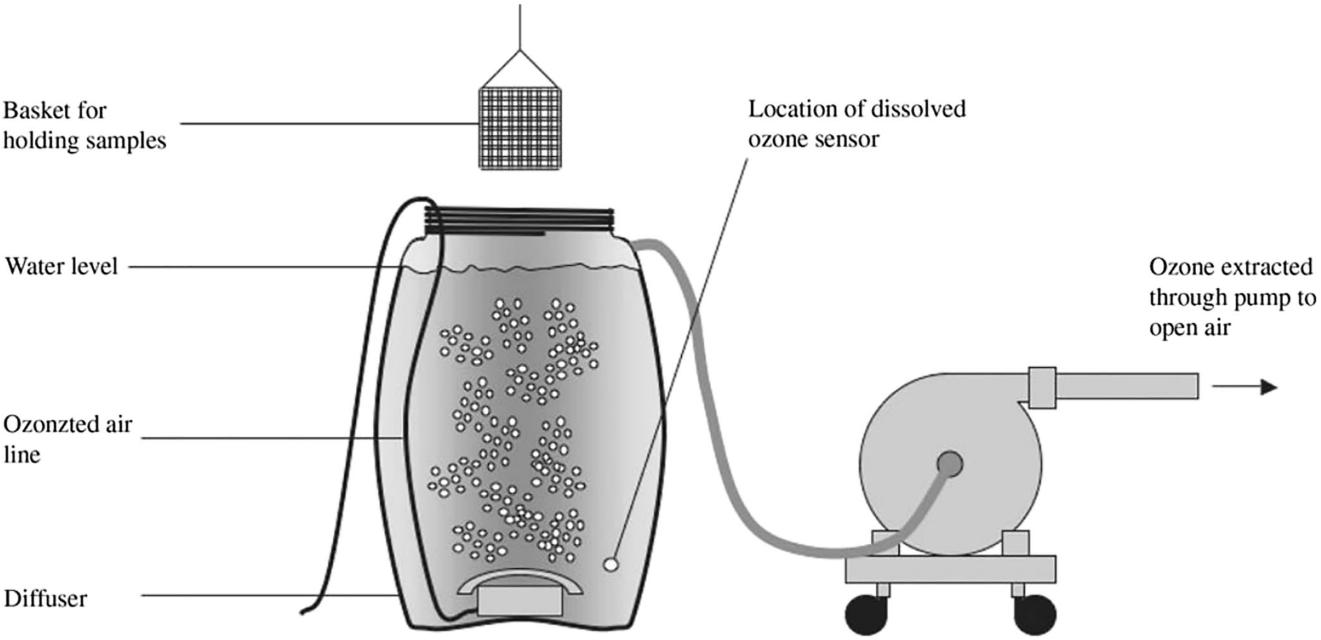

Fig. 3 Ozone sanitization system used for the treatment of precut bell pepper [65]

containing ozone and its extensive corrosive effect on metal surfaces such as fittings, pipes, and fans in a food processing plant have made its integration in the industry rather challenging [23]. This classification can be thus used for selection of materials for process equipment that has to be exposed to ozone.

Mechanism of Antimicrobial Action of Ozone

Ozone has been reported to exhibit a broad spectrum of antimicrobial activity that encompasses bacteria, fungi, viruses, mycotoxins, protozoa, and spore cells [67]. It has been found to be more effective than chlorine in inactivating *Bacillus subtilis* spores [15]. Ozone decomposes in water into free radicals, of an even higher oxidation potential than ozone, like, hydroxyl ($H\dot{O}$), hydroperoxy ($H\dot{O}_2$), and superoxide radicals (O_2^-). The high oxidizing power of these free radicals is believed to be responsible for the high reactivity of ozone [80]. The sanitizing effects of ozone are a complex process which includes the action of ozone on various cell walls and membrane constituents. The cell wall is subjected to lysis under high oxidation potential of ozone. Ozone has been shown to attack a number of bacterial constituents like unsaturated lipids, proteins, and respiratory enzymes in cell membranes, enzymes, peptidoglycans, and nucleic acids in the cytoplasm, as well as peptidoglycans and proteins in spore coats and virus capsids. It has been reported that ozone primarily attacks the double bonds of unsaturated membrane lipids, sulfhydryl groups of membrane-bound enzymes, and glycoproteins and glycolipids, killing bacteria by lysis mechanism [67]. Ozone-treated *E. coli* showed induction followed by inactivation of catalase (CAT) and superoxide dismutase (SOD) activity [138]. Upon the action of ozone on spores, it has been suggested from transmission electron microscope results that ozone degrades the outer spore component of the cells and thus exposes the core and the cortex to the action of ozone [38, 67]. It was also reported that ozone damages the ability of the spores to germinate which may be caused by the damage induced by ozone to the inner spore membranes [142]. Lysis of bacterial cell walls, probably as a result of oxidative stress, caused by ozone is a quicker inactivation mechanism as

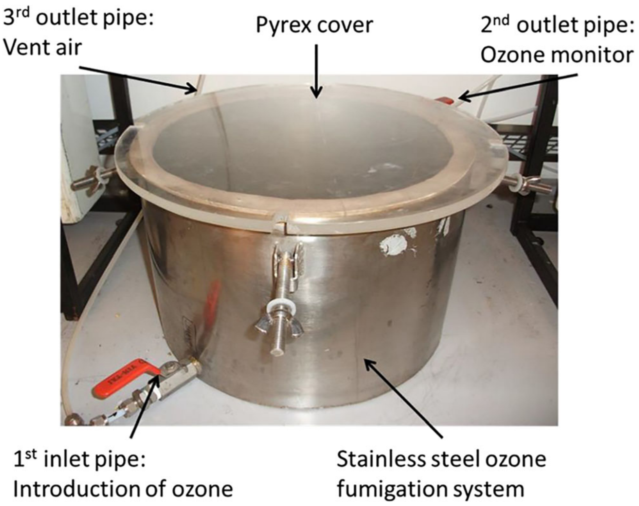

Fig. 4 Ozone fumigation system [136]

Table 2 Compatibility of materials with ozone (source: [25])

Material	Rating	Material	Rating
ABS plastic	Good	Natural rubber	Severe effect
Aluminum	Good	Neoprene	Fair
Brass	Good	Polysulfide	Good
Bronze	Good	Nylon	Severe effect
Cast iron	Fair	Polyurethane, millable	Excellent
Copper	Good	PVC	Good
CPVC	Excellent	Silicone	Excellent
Durachlor-51	Excellent	Stainless steel-304	Good/excellent
EPDM	Excellent up to 100 °F	Stainless steel-316	Excellent
Ethylene propylene	Excellent	Steel (mild, HSLA)	Poor
Fluorosilicone	Excellent	Polyacrylate	Good
Galvanized steel	In water (fair), in air (excellent)	Polyamide	Not recommended
Glass	Excellent	Polycarbonate	Excellent
HDPE	Excellent	Polyethylene	In water (good), in air (fair)
LDPE	Good	Polypropylene	Fair
Magnesium	Poor	Titanium	Excellent
Vamac	Excellent	Zinc	Poor

Excellent—no effect; good—minor effects; fair—breakdown within a week; poor—immediate breakdown

compared to other mechanisms, most of which depend on the permeation of the sanitizing agent through the cell walls to be effective [100]. It is also noteworthy that sanitization achieved by cell lysis does not create any instance of microbial resistance to ozone disinfection [100]. In 1997, ozone was approved for use in food processing by the United States Food & Drug Administration (FDA) and was thus affirmed a GRAS (generally recognized as safe) status for direct contact with foods by a panel of experts [47]. In 2001, it was approved as an antimicrobial food additive by the FDA [37]. Since then, the ruling has triggered a widespread interest in ozone as an antimicrobial agent and a direct food additive.

Factors Affecting the Sanitizing Power of Ozone

Ozone can be used in gaseous as well as in aqueous form for the postharvest treatment of fruits and vegetables. In the case of aqueous ozone treatment of fruits and vegetables, owing to a short half-life of the gas, the effects of environmental variables have been found to have a significant effect as compared to gaseous ozone. The efficiency of ozone in reducing the microbial load and affecting the quality of a fresh product depends upon a number of factors described under the succeeding headings.

Nature of the Product

The nature of a product has a great influence on the efficiency of ozone treatment. Products having a smooth surface exhibit better sanitizing efficiency with ozone as microbes entrapped in the rough surfaces are not exposed to the biolytic activity of ozone. These results were confirmed by experiments conducted by Achen and Yousef [1], where they observed that the sanitizing power of aqueous ozone was less in the rough stem calyx region of apples as compared to the smooth surfaces. Antimicrobial efficacy of ozonated water washing for green onions, grape tomatoes, and green leaf lettuces was also reported to be significantly dependent on their surface structures [141]. Surface characteristics such as cracks, texture, or hydrophobic tendency also affect the efficiency of ozone.

Mode of Ozone Delivery to the Product

Delivery methods that give ozone more contact time with the target microbial population increase its sanitizing efficiency. Achen and Yousef [1] compared the efficiencies of ozonated water in reducing the microbial count of inoculated apples by dipping them in ozonated water and by washing them in bubbling ozone gas through water. Apples were agitated to ensure full immersion and exposure to treatment water. It was found that the sanitization treatment of inoculated apples was more efficient when ozone was bubbled through the water than by dipping them in preozonated water. Similar results were confirmed by Kim et al. [69] when they treated lettuce with aqueous ozone. Bubbling gaseous ozone in water was observed to be the most efficient method of ozonation [69]. Some studies have also examined washing fresh vegetables with ozonated water applied via fine bubbles and ultra-fine bubbles with strong mechanical action. It was observed that with an increase in inlet ozone concentration, the average particle diameter of fine bubbles decreased, thus providing a significantly higher interfacial area per unit volume which results in higher diffusion of ozone toward the target material. The maximum eradication rate and a higher ozone utilization efficiency for inactivation of *B. subtilis* spores were reported for an average microbubble diameter of 49.7 μm at an ozone concentration of 140 mg/L [146]. In another example, Ushida et al. [133] studied the effects of washing fresh vegetables by ozonated and chlorinated water applied through fine and ultra-fine bubbles in an alternating flow demonstrating strong mechanical action on the produce. The authors reported that ultra-fine ozone-rich bubbles applied through an alternating flow showed a significantly efficient sanitizing effect (3.7 log CFU/g) as compared to sodium hypochlorite (4.6 log CFU/g) for Chinese cabbage. It was concluded that stronger washing effects may be obtained by applying dissolved ozone through ultra-fine bubble mixtures combined with an alternating flow.

Process Temperature

Solubility of ozone is negatively dependent on temperature and the gas becomes literally insoluble above 60 °C [111]. However, bactericidal effects of ozonated water have been observed to improve with the increase in temperature. At higher temperatures, aqueous ozone disintegrates into free radicals very rapidly, thus being available for a short time which may be a reason for increase in antimicrobial activity with temperature for short exposures. Xu and Wu [141] reported an improved inactivation of *Salmonella* from the surfaces of tomatoes and lettuce by washing them with ozonated water at mild heated temperature (50 °C). A 4.14 log reduction was reported at 50 °C for *Salmonella* inactivation as compared to 2.62 log reduction at 4 °C in the case of tomatoes [141]. Similarly, Kim [68] reported that ozone applied at a higher temperature reduced more contaminants as compared to ozone applied at refrigeration temperatures, although in a number of produce, temperature has not been observed to significantly affect the microbial inactivation. For example, in the treatment of apples at 4, 22, and 45 °C, no significant differences in the reduction of *E. coli* count were reported [1]. The increase in reactivity of ozone may thus be compensated with the decrease in its stability with increase in water temperature.

Process pH

Ozone is less stable at higher pH values, which is because of the availability of more OH^- ions at higher pH, that initiate the decay of ozone. Karaca and Velioglu [62] suggested that at lower pH of water, the stability and efficiency of aqueous ozone may be enhanced. At high pH, with the presence of more OH^- ions, ozone decomposition rate is changed substantially, producing numerous radical species.

$$O_3+OH^{-}\text{-------}H\dot{O}_2+\dot{O}_2$$

In this sense, ozone applied at lower pH (2.6) was reported to significantly lower (3.49 log reduction) the *Salmonella* count on green onions as compared to ozone applied in deionized water (2.59 log reduction). The pH effects were also observed to be dependent on the type of produce [141]. Patil et al. [101] also studied the effects of pH (levels of 3.0, 3.5, 4.0, 4.5, and 5.0) on the ozone inactivation kinetics in apple juice. The studies revealed that for an initial inoculation of 10^6 CFU/mL in juice, ozone treatment duration for a 5 log reduction at pH 3.0 was faster (4 min) than at pH 5.0 (18 min).

Relative Humidity of the Storage Space

The efficacy of ozone gas in sanitizing fruits and vegetables is strongly dependent on the relative humidity (RH) value. RH value of 90–95% in controlled atmosphere storage rooms is considered as optimum. In general, ozone has been reported to lose its bactericidal effect below RH of 50%. The presence of moisture either in air or in food material solubilizes ozone and thus enhances the contact between the gas and grain. Han et al. [50] observed that ozone gas exhibits strong sanitizing effects when the RH levels are generally over 80%. At high RH, the microbial effects of ozone gas have been found to be higher than other gaseous disinfectants such as propylene oxide and ethylene oxide [140], but lower than that of chlorine dioxide gas [50].

Ozone Demand of Water

Organic matter present in water may exhibit high ozone demand and subsequently compete with the microbes for ozone [67]. In the presence of organic matter, dissolved ozone is rapidly consumed and transformed to oxygen. The initial amount of ozone that is consumed by the water itself and produces no bactericidal effects on the produce is referred to as the ozone demand of water. Furthermore, reaction of ozone with organic matter can produce undesirable by-products that can eventually interfere with the organoleptic properties of a food product, for which the sanitization was intended [67]. Hence, organic matter free water should be used for ozonation of fruits and vegetables.

Kinetic Modeling of Ozone Gas

While designing a sanitization step for a particular product, both the inactivation capability and characteristics of the sanitizer must be properly considered. Even a potential sanitizer would not provide the desired level of sanitization if the microbes and the sanitizing agent are not in contact for a sufficient period of time [118]. As such, it is important to determine the decomposition kinetics of ozone, so as to select the initial concentration to be applied to effectively achieve the required level of sanitization. Understanding the behavior of ozone gas is very fundamental to use it as a fumigant on a commercial scale [51]. When applied as a gas, its behavior with the porous medium is studied by estimating the saturation time of the medium, half-life, and decay rate. The reaction kinetics mentioned here follow from Paes et al. [96] who studied the gas behavior in the case of wheat flour. The authors ozonated 3.5 kg wheat flour at concentrations of 0.54, 1.07, 1.61, and 2.14 mg/L inside a prototype consisting of a cylinder and a helical mixing shaft. The saturation time was determined by quantifying the ozone levels inside the cylinder after every 20 min until the levels inside reached a constant value. To determine the reaction kinetics (half-life and decay rate), the vents were closed and the gas was allowed to react with the porous medium for a time of 60 s (decomposition time). The residual concentrations of ozone were fitted to zero-, first-, and second-order models. The models were further adjusted by employing regression analysis, with determination coefficient (R^2) as a selection parameter. The decay rate (k) was estimated from the slope of the model and the half-life ($t_{1/2}$) from the equation:

$$t_{1/2}=\frac{\ln 2}{k}$$

It was observed that the first-order integrated and linearized model provided the best determination coefficient for the applied concentrations ($0.84 < R^2 < 0.93$). The first-order reaction can be illustrated as:

$$\ln C=\ln C_0-kt$$

In this model, the average values of decay rate were 0.23 ± 0.008 min^{-1} and the average half-life time was 3.02 ± 0.081 min. Similarly, various researchers have authenticated the use of the first-order model for evaluating the decomposition kinetics of ozone gas [4, 51]. The decay rate and half-life of ozone depend not only on the temperature factor [4] but also on the characteristics of the product. In the case of wheat flour, more surface area of the product is available as compared to whole wheat grains for reaction, and thus, the gas is decomposed rapidly yielding a low half-life and, subsequently, a high decay rate. Supporting this hypothesis, Alencar et al. [4] reported a decay rate and half-life values of 0.094 min^{-1} and up to 7.7 min, respectively, in the case of whole peanut grains.

On the other hand, ozone gas dissolved in water decomposes more rapidly than in gaseous state [55]. This is due to the fact that the instantaneous ozone demand of water is greater than the gaseous phase. Initial ozone demand increases with the increase in the organic load of water. Almost 50% of ozone destroys in 20 min at 20 °C in tap or distilled water, whereas in double distilled water, only 10% breaks down after 85 min at the same temperature [55]. The degradation kinetics of ozone in aqueous medium follows the first-order kinetics [64]. Selma et al. [116] evaluated the effects of aqueous ozone on the inactivation of *Yersinia enterocolitica* and the reduction of natural flora on potatoes and developed a first-order kinetic model to estimate the decomposition kinetics of aqueous ozone. Consequently, if C_0 is the ozone dose applied (mg/L), the residual ozone in the solution at any time t is given by C_t as:

$$C_t=(C_0-D)\exp\left(-k^{*}t\right)$$

where k^* is the decay rate (min^{-1}) and D is the instantaneous ozone demand of water. A number of other disinfection kinetic models are given in Table 3. Shynkaryk et al. [118] developed a mathematical model to evaluate the diffusion of ozone into the interior of green leafy vegetables. The reaction rate of ozone was experimentally determined for a lettuce leaf and used in numerical simulation to evaluate penetration of ozone within the lettuce leaves. It was assumed that within the small pores in the produce, mass transport of ozone will be exclusively diffusive which was stated as:

$$\frac{\partial c}{\partial t}+\nabla(-D\nabla c)=-R_{\mathrm{d}}$$

where c is the concentration, t is the time, D is the diffusivity, ∇ is the gradient operator, and the term $R_{\mathrm{d}} = k_{\mathrm{d}}c$ is associated with the first-order ozone self-decomposition rate. The boundary condition for ozone decomposition rate on the foliage surface of lettuce was given as

$$\vec{n}(-D\nabla c)=-R_{\mathrm{L}}$$

Where $\vec{n}$ is the unit normal vector. Assuming γ to be the probability of uptake of ozone, the flux of incident ozone molecules, F was defined as

$$F=R_{\mathrm{L}}/\gamma$$

However, for an ideal gas mixture and low temperature conditions, F can be defined by the Hertz–Knudsen equation as

$$F=\frac{p_i}{\sqrt{2\pi mkT}}$$

where $p_i = c_ikT$ is the partial pressure of ozone gas (Pa), m is the molecular mass (kg), c_i is the ozone gas concentration, T is the temperature, and k is the Boltzmann constant. The problem was simulated using COMSOL software and was verified with experimental values. It was observed that the penetration

Table 3 Disinfection kinetic models for dissolved ozone

Name	Model	Kinetic parameters	Reference(s)
Modified Chick	$\log(S_t) = -k\frac{C_0-D}{k^*}\left[1-\exp\left(-k^*t\right)\right]$	k, k^*, D	Kaymak [64]
Modified Chick–Watson	$\log(S_t) = -k\frac{(C_0-D)^q}{qk^*}\left[1-\exp\left(-qk^*t\right)\right]$	k, k^*, q, D	Cho et al. [22]
Modified Chick power law	$\log(S_t) = \frac{-1}{p-1}\log\{1+\frac{(p-1)k(C_0-D)N_0(p-1)}{k^*}\left[1-\exp\left(-k^*t\right)\right]\}$	k, k^*, p, D	Kaymak [64]
Modified Chick–Watson power law	$\log(S_t) = \frac{-1}{p-1}\log\{1+\frac{(p-1)k(C_0-D)^qN_0(p-1)}{qk^*}\left[1-\exp\left(-qk^*t\right)\right]\}$	k, k^*, q, p, D	Kaymak [64]
Modified multiple target	$\log(S_t) = \log\left(1-\left[1-\exp\left\{\frac{k(C_0-D)[\exp\left(-k^*t\right)-1}{k^*}\right\}\right]^{n_c}\right)$	k, k^*, n_c, D	Kaymak [64]

S_t—survival ratio; C_0—initial applied ozone dose; D—instantaneous ozone demand; $k*$—ozone decay rate; t—time; k, p, q—rate parameters; n_c—number of critical targets

depth of aqueous ozone on lettuce leaves was only a few millimeters due to significant time required for diffusion until which the residual ozone degrades.

Optimization of Ozonation Process Variables

Although ozone has successfully emerged as one of the potent alternatives for fresh and fresh-cut produce sanitization, discrepancies in results are still reported in the literature because of involvement of a great number of variables including ozone concentration and duration time, pH of water, temperature, method of application, nature of produce, and microbes [130]. The optimization of process variables has been preferably carried out using the response surface regression procedure using a suitable design based on the number and levels of process variables and numerical optimization to arrive at the most feasible combination. The optimization is mainly intended to obtain higher degrees of microbial log reductions and a minimal effect on quality parameters. Ölmez and Akbas [91] optimized the process parameters (ozone concentration, exposure time, and temperature) for sanitizing fresh-cut green lettuce with aqueous ozone, using the response surface regression model. It was observed that for a temperature range of 10–26 °C, the effect of temperature on the sanitizing efficiency of aqueous ozone was nonsignificant. Although higher sanitizing efficiency has been reported at higher temperatures, ozone becomes unstable with increase in temperature, hence compensating for the effect. The optimized conditions obtained were 2 ppm of ozone concentration for an exposure time of 2 min to achieve the desired results. Similarly, the effect of ozone treatment (2 to 8 mg/L), RH (60 to 90%), and treatment time (10 to 40 min) was studied by Han et al. [50] on the inactivation of *E. coli* on green peppers using response surface methodology. A three-factor Box–Behnken experimental plan was designed and microbial log reduction was measured as a response. Among the three factors, the effect of ozone gas concentration on bacterial inactivation was the greatest, while the effect of RH was the least. The interaction between ozone gas concentration and RH exhibited a significant and synergistic effect ($P < 0.05$) on the total microbial log reductions.

In addition to this, a central composite design with five central points was used by de Souza et al. [29] to optimize ozone concentration, exposure time, and temperature while optimizing aqueous ozone treatment for pesticide removal from carrots. It was noted that temperature did not have a significant effect on the process, while ozone concentration and exposure time significantly affected the pesticide removal. O_3 application at optimized conditions of 5 and 10 mg/L were successful in removing more than 80% of the pesticide content. This indicates that optimization of ozone process parameters is a critical step in maximizing ozone use efficiency in a food processing line.

Cost Analysis

Initial capital investment required for setting up an ozonation treatment system in a food processing plant may be higher than conventional methods of sanitization or fumigation, but it has been found to be reasonably economical for long-term applications in developed countries [92]. The raw materials required to generate ozone is atmospheric oxygen, which is inexhaustible and abundantly available and has no environmental implications, and thus, the running costs are low as electrical energy cost required to produce ozone is the main component of cost expenditure. The ozone generator is the primary power drawing component in a typical food processing plant involving ozone sanitization, and the power required depends mainly on the gas output capacity of the generator and also varies from manufacturer to manufacturer. The power consumption varies from 90 W for a generator with a rated capacity of 10 g/h (L10G, Faraday Ozone, Coimbatore, India) to 32.35 kW for a commercially used four-quad (16 chamber) ozone generator having a rated capacity of 1000 g/h (OzoBlast, O_3CO, Aberdeen, ID, USA). The power rating in oxygen concentrators/air treatment systems also varies with the capacity of oxygen flow. For example, an oxygen concentrator (OXY-5, Faraday Ozone, Coimbatore, India) with an outlet gas flow rate of 5 lpm has a rated power consumption of 320 W, which can go up to 5.6 kW in a concentrator giving 100 lpm of highly concentrated oxygen (OXY-100, Faraday Ozone, Coimbatore, India). However, it is pertinent to refer to the manufacturer's specifications of the product to select a system that best fits to a processor's needs. Pertaining to the nature of operations, continuous large-scale treatment systems have been reported to be more economical than batch-type processes due to a higher process rate. Campabadal [20] reported that while fumigating 1272 MT of grain in a processing facility using 1000 g/h of ozone, a continuous flow treatment system incurred the lowest cost (1.21 $/MT) as compared to fixed bed ozonation (1.33 $/MT) and semicontinuous counterflow ozonation (2.72 $/MT). The treatment cost for the most economical ozone process was still 35% higher than the treatment cost for contract phosphine fumigation of 0.78 $/MT, but with a 100% insect mortality rate. However, in aqueous ozone applications, significantly lower gas output generators are required. Although numerous studies have reported aqueous ozone sanitization of fresh and fresh-cut produce, cost analysis is needed to test the economic feasibility of using aqueous ozone at an industrial scale. In addition, during a cost analysis study of a mobile ozone surface sanitation system performed by a food processing facility, it was observed that the facility normally spent $6000 per year in chemical costs for sanitization of surfaces. With the use of ozone as a surface disinfectant, its wastewater disposal was lowered from 15,000 to 6000 gal/day amounting to a total annual savings of $18,960 [112].

Role of Ozone in Postharvest Management of Fruits and Vegetables

Fruits and vegetables are very susceptible to spoilage induced by microorganisms, thus reducing their postharvest shelf life. Ozone has demonstrated strong sanitizing properties which are consequently useful in enhancing shelf life and keeping the quality of fresh produce. Ozone, however, also interferes with the product quality and can have either desirable or an undesirable effect. It has been observed to affect sensory (aroma, color, texture, weight loss) as well as nutritional (vitamins, antioxidant capacity, bioactive compounds) attributes in certain foods depending upon the dose applied. Several researchers have studied the effects of ozonation on the biolytic activity and quality parameters as discussed below.

Biolytic Activity of Ozone

The fungicidal activity of ozone has been confirmed in a number of studies [11, 41, 83, 98, 114]. The mechanism of fungicidal activity of ozone can be attributed to its capability to cause membrane integrity damage. Different species have been found to respond to ozone differently. For instance, Palou et al. [98] compared the effects of ozone on the growth of *Penicillium digitatum* and *Penicillium italicum* and found that while the former was resistant, the growth of the latter was inhibited by the treatment with ozone. Similarly, gaseous ozone was more efficient than aqueous ozone in reducing toxins, while the opposite was observed for mold growth [149]. Moreover, fungal decay caused by *Botrytis cinerea* in blackberries was effectively reduced by gaseous ozone [11]. In the case of strawberries, earlier studies [124] found out that ozone was rather ineffective in reducing *Botrytis* and *Rhizopus* rot. However, Nadas et al. [86] reported that cold storage of strawberries enriched with gaseous ozone (1.5 μL O_3/L) reduced decay caused by *B. cinerea* significantly as compared to control samples. This variability in results can be attributed to the difference in the treatment parameters and also to the precision of recording measurements. However, studies supporting the efficiency of ozone in controlling spoilage-causing microbes in strawberries overpower the ones against it [13, 14, 114]. Furthermore, Spotts and Cervantes [125] while trying to control fungal rot caused by *Penicillium expansum* in pears observed that ozonated water treatment (3.1 μg O_3/mL for 5 s) was rather ineffective in controlling the already emerged rot. However, it succeeded in reducing lesion diameters as compared to the control samples. Moreover, the germination of *B. cinerea*, *Mucor piriformis*, and *P. expansum* spores was inhibited after treating the produce with 0.1–4 μg O_3/mL. Peaches inoculated with *Monilinia fructicola*, *B. cinerea*, *M. piriformis*, or *P. expansum* were subjected to gaseous ozone at a concentration of 0.3 ppm (v/v) in the storage atmosphere for 4 weeks at 5 °C and 90% (RH). It was observed that sporulation and the external mycelial growth of all the fungal species were affected as a result of ozone. However, the treatment was not effective in controlling either the incidence or decay caused by these fungi, except for *M. fructicola* [99].

The bactericidal effects of ozone both in aqueous and gaseous phases on Gram-positive and Gram-negative bacteria as well as on vegetative- and spore-forming cells have been confirmed by numerous studies [17, 104]. It can be generalized that the antimicrobial efficacy of ozone varies to a large extent depending mainly on experimental conditions. It has been observed that application of ozone in gaseous phase is preferred to aqueous phase for enhancing the shelf life of fruits which may be due to the ability of ozone gas to control ethylene production from fresh fruits. The comparison of aqueous and gaseous ozone for its application to fresh fruits is illustrated in Table 4 and Table 5, respectively. The nature of the bacterial population also influences the efficacy of treatment. A 6-log reduction in *Salmonella enteritidis* counts in distilled water at 1.5 ppm ozone concentration was observed by Dave et al. [28]. In another study, Restaino et al. [110] demonstrated

Table 4 Effect of aqueous ozone on some common fruits

Type of produce	Treatment			Major findings	Reference(s)
	Concentration	Exposure time	Environment		
Apple	21–28 mg/L	1–5 min	2, 22, and 45 °C	• 3.7 LR of *E. coli* O157:H7 were achieved	Achen and Yousef [1]
	1.4 mg/L	5, 10 min		• 2.13 LR were obtained • Ethylene (↓), polyphenol oxidase and peroxidase activities (↓), total phenol content (↓) malondialdehyde (↓), antioxidant activity(↑)	Liu et al. [79]
Banana	0.36 mg/L	10 min	25 °C for 12 days (S)	• Weight loss (↓), TSS (↓), titratable acidity (~), fungal-induced lesions (↓), texture (~)	Alencar et al. [5]
Watermelon	0.4 μL/L	–	–	• No changes in total bacterial count, color (↓)	Fonseca and Rushing [39]

(S)—storage conditions, *LR*—log reductions, *(↑)*—increase, *(↓)*—decrease, *(~)*—no change, *TSS*—total soluble solids

that Gram-negative bacteria (*E. coli*, *Y. enterocolitica*) are more resistant to ozone in the water phase than Gram-positive bacteria (*L. monocytogenes*). This resistance has been attributed by some scientists to the higher peptidoglycan content in the cell walls of the former class [69, 134]. Crowe et al. [26] reported that aqueous ozone when sprayed over blueberries reduced bacterial counts of the genus *Pseudomonas* by 2.80 log reductions. In the case of raspberries, Bialka and Demirci [13, 14] observed that aqueous ozone was more effective in reducing counts of *Salmonella* (4.5 log reductions) and *E. coli* O157:H7 (5.6 log reductions) than gaseous ozone (1.6 and 2.6 log reductions, respectively). Aqueous ozone, on the other hand, was observed to reduce *E. coli* O157:H7 and *L. monocytogenes* to an undetectable level in strawberries during 9 days of storage at 4 °C [114]. In another similar set of experiments, strawberries inoculated with *Salmonella* and *E. coli* O157:H7 were treated with either gaseous [13] or aqueous [14] ozone. The results obtained showed that aqueous ozone washing at 20 °C was more effective with maximum log reductions of 3.3 and 2.9 for *Salmonella* and *E. coli* O157:H7, respectively, as compared to gaseous treatment (0.9 and 1.8 log reductions, respectively). Similarly, ozone was found to be effective in reducing *Y. enterocolitica* population in potatoes by 1.6 log units ([116]); *P. carotovorum* population in carrots by 1.5 log units ([52]); mesophilic bacteria in unwaxed cantaloupes by 4–5 log units ([114]); complete reduction of *S. enteritidis* in cherry tomatoes ([27]); lactic, anaerobic, and coliform bacteria in minimally processed potato strips by 3.29, 1.2, and 3.0 log reductions, respectively ([12]); and *E. coli* O157:H7 in minimally processed carrots by 1.8 log units (aqueous washing) and by 2.64 log units (gaseous exposure) [119].

Ozone has also been reported to inactivate yeasts and molds [34]. However, molds seem to be more resistant to ozone than yeasts. In a study conducted by Restaino et al. [110], it was observed that ozonated water reduced *Zygosaccharomyces bailii* and *Candida albicans* by more than 4.5 log reductions, whereas less than 1 log reductions were recorded for *Aspergillus niger* when exposed for 5 min. In another set of experiments carried out by Sarig et al. [115] on table grapes, native populations of molds, yeasts, and bacteria were successfully eliminated by treating with 8 mg O_3/min. Similarly, Mlikota-Gabler et al. [84] observed that the efficiency of ozone in controlling gray molds in table grapes depends not only on the ozone parameters but also on the cultivars' natural resistance to the molds. The authors reported that fumigating table grapes with ozone (5000 μL/L O_3 for 60 min) reduced the incidence of gray molds by about 65% in 'Redglobe' and by 50% in 'Autumn Seedless' and 'Black Seedless' cultivars; 1–2 log reductions in yeasts and molds were recorded in sweet cherries treated with aqueous ozone as compared to control samples [74]. Similarly, Palou et al. [98] observed that the incidence of blue molds in lemons was delayed after treatment with 0.3 ppm ozone during 3 weeks of storage at 4.5 °C. When compared to chlorine washing, ozone has been reported to have even better bactericidal effects. For example, minimally processed bell pepper after treatment with gaseous ozone proved to be a better sanitizer than chlorine in the reduction of total microbial count [56]. Whether ozone is to be used as a gas or in aqueous phase largely depends on the product commodity and the purpose of storage; however, owing to the higher stability of ozone in gaseous form, it sometimes yields better results. The use of gaseous and aqueous ozone in enhancing shelf life and keeping the quality of fresh vegetables is shown in Table 5 and Table 6, respectively.

Effect of Ozone on Quality Attributes of Fruits and Vegetables

Different doses of ozone have a significant effect on both the nutritional and sensory attributes of ozonated fruits and vegetables for the fresh market. The impact of ozonation treatment on the wholesomeness of fresh and fresh-cut produce is discussed below.

Table 5 Effect of gaseous ozone on some common fruits

Type of produce	Treatment			Major findings	Reference(s)
	Concentration	Exposure period	Environment		
Apple	0.05–0.4 µL/L	107 days	0 °C and 90–95% RH	• Ethylene (↓), firmness (~), total soluble solids (~) titratable acidity (~) • No tissue injuries from oxidative stress were reported	Skog and Chu [121]
Dried figs	1, 5, and 10 ppm	3–5 h	20 °C	• 38 and 72% reduction in aerobic and mesophilic count, respectively • 5 ppm for 3 h eliminated coliform colonies	Öztekin et al. [95]
	0.1, 0.5, and 1 ppm	6 h	20 °C and 70% RH	• 3.5 LR (at 1 ppm) in *E. coli* and *B. cereus* counts • Sensory attributes (flavor, rancidity, appearance, sweetness and overall palatability) (~), physiochemical attributes (moisture content, pH or color (~)	Akbas and Ozdemir [3]
Kiwifruit	150 ppb during the day and 180 ppb during the night	42 days	0 and 18 °C (S)	• Firmness (↑), soluble solid content (↓), color change (↑), microbial load (~)	Goffi et al. [44]
Muskmelon	1.10 and 2.20 mg/L	30, 60, and 120 min	22 ± 2 °C and 75–80% RH. (S) for 5, 8, and 11 days in plastic bags with a sterile moist paper towel	• *Fusarium* rot development and neosolaniol (NEO) accumulation was significantly reduced • Lesion sizes reduced upon prolonged exposure	Hua-Li et al. [57]
Orange	0.3 ppm	3 weeks	4.5 °C	• Green mold appearance on preinoculated oranges was delayed by 1 week's time • Growth of both mycelia and conidia of *P. digitalum* and *P. italicum* were inhibited • Ethylene (↓)	Palou et al. [98]
Papaya	0.05–5.8 ppm	0.5–24 h	25 ± 3 °C and 70 ± 5% RH	• Up to 99.7% reduction in mesophilic bacteria. Peel color (~), titrable acidity (~), firmness (~), total solids (↑) weight loss (↓),	Kying and Ali [76]
Pear	0.05–0.4 µL/L	107 days	0 °C and 90–95% RH	• Ethylene (↓), firmness (~), total soluble solids (~), titratable acidity (~) • No tissue injuries from oxidative stress were reported	Skog and Chu [121]
Strawberry	0.35 ppm	3 days	2 °C 20 °C (S)	• 15% less fungal (*Botrytis cinerea)* decay on the 3rd day under treatment • After 4 days in storage, rot conditions were similar to untreated • Sugar (↓), ascorbic acid (↓), volatile esters (aroma) (40%↓)	Pérez et al. [102]
Table grapes	0.3 ppm	7 weeks		• Nesting and sporulation of fungus were prevented • Gray mold incidence was not significantly reduced	Sarig et al. [115]
	2 ppm	72 days	5 °C	• Decay percentage (↓), firmness (↓) (intermittently treated samples showed better firmness), flavor (↓)	Cayuela et al. [21]
	2500–5000 µL/L	1 hour	7 days at 15 °C or 28 days at 0.5 °C (S)	• Gray molds (50% ↓) • Longitudinal lesions because of oxidative stress appeared	Mlikota-Gabler et al. [84]

(S)—storage conditions, *LR*—log reductions, *(↑)*—increase, *(↓)*—decrease, *(~)*—no change

Impact on Nutritional Components

The intent of food processing can be very diverse. Foods may be processed to extend their shelf life and to enhance or keep the quality or to improve its digestibility, to increase its palatability or texture, to give it a ready-to-use status, to create new forms of foods, to remove inedible parts, to destroy toxins and antinutritional components, or to eliminate spoilage-causing and pathogenic microorganisms. Usually, in this step, the nutritional content of foods gets reduced. But it can be regarded as a necessary price to pay to ensure their safety [32]. Thus, it becomes important to optimize between safety and retention of nutritional factors in foods. With ozone being a strong oxidizing agent, it would be expected to cause alterations in the nutritional levels of foods after prolonged exposure to high doses. However, it was reported that the impacts of ozone on food materials are restricted to surface only and it does not penetrate into the bulk of the material [75, 118]. Hence, any negative impact on nutrient content caused by ozone can be assumed to be restricted to surface only. The impact of ozone

Table 6 Effect of aqueous ozone on some common vegetables

Type of produce	Treatment			Major findings	Reference(s)
	Concentration	Exposure time	Environment		
Carrot	0–10 mg/L	120 min		• Weight loss (~), firmness (~), color (~) • Pesticides (↓), shelf life (↑)	de Souza et al. [30]
Bell pepper	1–3 mg/L	1–5 min	5 ± 0.5 °C, 85% ± 5% RH (S)	• Microbial load reduced to undetectable levels up to 6 days of storage with 3 mg/L, weight loss (~), texture (~), color (~), microbial load (↓), TSS (~)	Ummat et al. [132]
Cauliflower	0.31–0.35 ppm	15 min	3 °C for 18 days (S)	• 1.8 and 1.88 LR in TPC and *E. coli* count respectively. Shelf life (↑), color (↑)	Sothornvit [123]
Celery	0.03, 0.08, and 0.18 ppm	5 min	4 °C (S)	• 1.69 LR in bacterial count, color (~), total sugar (~)	Zhang et al. [145]
Green asparagus	1 mg/L	30 min	3 °C in MA (S)	• Lignin, cellulose and hemicellulose accumulation (↓), antioxidant activities (↑), shelf life (↑)	An et al. [8]
Lettuce	0.5–16.5 ppm	1–5 min		• *S. sonnei* population was reduced by 5.6 log CFU/mL	Selma et al. [117]
	5.2, 9.7, 16.5 mg/L (preceded by chlorine dioxide 10 mg/L for 10 min)	1, 5, 10, 15 min	22 °C and 80% RH	• 1.42 LR were achieved for *E. coli* O157:H7 • Color (↓)	Singh et al. [119]
	1.3, 2 mM	5 min		• 3.9 and 4.6 LR were observed for mesophilic and psychotrophic bacteria respectively.	Kim et al. [69]
	0.15, 0.25 0.55 and 0.65 ppm,	1, 5 and 10 min	50, 22 and 4 °C, pH levels from 5.60 ± 0.03 to 2.64 ± 0.02	• 2.24 LR (at 50 °C), 2.53 LR (at 2.64 pH), 1.87 LR (at 4 °C) and 1.56 LR (at normal pH)	Xu and Wu [141]
	2.5, 5.0, and 7.5 mg/L	1–5 h	4 °C for 25 days (S)	• LR observed in aerobic plate count • Shelf life (↑) • Color (↓)	Garcia et al. [42]
Mushroom	1, 3, and 5 ppm at 22 °C	0.5, 1, 3, and 5 min	22 °C 15 °C for 10 days (S)	• 0.94 LR for *E. Coli* O157:H7 and no reductions in *L. monocytogenes*	Yuk et al. [143]
Potato strips	20 mg/L		4 °C for 14 days in MAP/vacuum (S)	• Coliforms (↓), anaerobic bacteria (↓), color (~) (up to 5 days), aroma and texture (~) (for 14 days), shelf life (↑), nonenzymatic browning (↑)	Beltrán et al. [12]
Potatoes	5 ppm	30 s to 5 min		• 1.5, 1.1, 0.8, and 0.7 log reductions were reported in the counts of coliforms, mesophilic, *L. monocytogenes*, and psychotrophic bacteria, respectively	Selma et al. [116]
Spinach	5 ppm	3 min	Room temperature	• 1.22 and 1.4 LR in *E. coli* and *L. monocytogenes* count, respectively; 0.88 LR in yeast and fungal count	Rahman et al. [108]
Tomatoes	0.15, 0.25, 0.55, and 0.65 ppm	1, 5, and 10 min	50, 22, and 4 °C, pH levels from 5.60 ± 0.03 to 2.64 ± 0.02	• 4.93 LR (at 2.64 pH), 3.11 LR (at normal pH), 4.14 (at 50 °C) and 2.62 (at 4 °C) LR were observed for *S. enterica typhimurium*	Xu and Wu [141]

on product quality and nutritional value has been reported by many investigators ([1, 11, 13, 14, 39, 49, 102, 121, 128, 131, 145]). Numerous studies have illustrated how ozone favorably or unfavorably affects the nutritional factors of foods.

Among vitamins, vitamins C and B_1 (thiamin), carotenoids, and folates are the most vulnerable under ozone treatment. Vitamins C and B_1 are often utilized as an indicator factor to monitor the effects of food processing technique over nutritional factors. Due to the high reactivity of ozone, it is quite difficult to predict the exact mechanism of reaction with these components. It oxidizes organic matter present in foods primarily by two pathways: by direct reaction or by producing free radicals. Both oxidative and nonoxidative mechanisms can be responsible for ascorbic acid degradation in foods ([127]). The effects of ozone on ascorbic acid do not usually follow a specific trend with the reduction varying from produce to produce and the method of ozone application. For example, Karaca and Velioglu [63] reported a 40% reduction in ascorbic acid contents in parsley after a gaseous ozone exposure of 950 ± 12 μL/L for 20 min. Ascorbic acid content decreased from 10.1 to 6 g/kg DM which was a significant reduction. Similarly, Keutgen and Pawelzik [66] reported a significant reduction in ascorbic acid levels of strawberries grown under ozone-enriched atmosphere with a concentration of 156 μg/m^3 for a period of 2 months. On the other hand, Ummat et al. [132] while treating bell pepper shreds with 2 mg/L aqueous ozone for 5 min observed just 3% change in ascorbic acid levels which was quite insignificant. Similarly, aqueous ozone treatment (3, 5, and 10 ppm concentrations for 5 min) of fresh-cut iceberg lettuce did not significantly affect its ascorbic acid content [73]. Moreover, washing lettuce with cold ozonated water (4–5 ppm) reduced losses in vitamin C and sugar content as compared to ozonated tap water [53]. Ascorbic acid losses were also reported in broccoli florets treated with gaseous ozone [148] and rocket leaves treated with aqueous ozone [81]. Naitoh and Shiga [88] treated wheat flour with 0.5–50 ppm ozone for an exposure time of 6 h, to reduce spoilage-causing microbes in the flour to be used for noodle making. The authors reported no change in riboflavin content of the flour, although some thiamin content was lost. Thus, it can be concluded that vitamin C content is greatly affected by gaseous as compared to aqueous ozone treatment, which can be partly attributed to a longer half-life of ozone in the gaseous phase.

Phenolics and other antioxidant compounds are assumed to serve as a natural substrate for oxidizing reactions of ozone treatment. The sensitivity of these compounds to oxidative reactions of ozone may be dependent on the type of compounds and their position in the food matrix. Karaca and Velioglu [63] reported a 12% reduction in total phenolic content of parsley after exposing it to 950 ± 12 μL/L ozone gas concentration for 20 min. A similar reduction of free phenolic content in ozone-treated (150 μL/L for 5 days) pumpkin leaves has also been reported [109]. Phenolic content of strawberries [7] and antioxidant potential of citrus leaves [60] were also reported to decrease after a gaseous ozone treatment. Moreover, Tzortzakis et al. [131] observed a negligible change in weight, antioxidant content, ethylene production, CO_2/O_2 exchange, vitamin C, and total phenols in tomato fruit when exposed to ozone concentrations between 0.005 and 1 μmol/mol at 13 °C and 95% RH. Aqueous ozone treatment of celery and strawberries did not alter total sugar content during storage [145].

There is no evidence in the literature that ozone treatment causes destruction of amino acids and fatty acids at the levels prescribed for food processing, nor has ozone been reported to interfere in the protein quality of food materials [31].

Impact on Sensory Attributes

Ozone has been reported to show both favorable and unfavorable effects on the sensory quality, depending upon the nature of food products and ozone concentration. Generally, below a concentration of 1 ppm, changes in sensory and chemical composition of a food product are rather insignificant. But such a low concentration cannot always yield the desired log reductions in microbial counts. On the other hand, high doses significantly deteriorate sensory qualities in most of the foods [3, 49, 89, 144, 147].

One of the most notable effects of ozone treatment is the oxidation of volatile components, resulting in loss of aroma. The effect of ozone on the volatile components of spices (ground black pepper and whole black peppercorn) was evaluated by Zhao and Cranston [147]. The authors reported a significant reduction in aroma in ground black pepper as compared to whole black peppercorn. These findings may be a result of better availability of volatile components in ground spices for oxidation with ozone. Losses in fruit aroma were also reported in strawberries stored in an ozone-enriched cold storage [86, 102]. Pérez et al. [102] reported a 40% reduction in volatile ester emission in strawberries. Furthermore, no changes in sensory quality were reported in ozone gas–treated onions [122].

Low doses of ozone did not cause changes in the color of several fruits and vegetables. For instance, the red color of blackberries was maintained for 12 days of storage at 2 °C after treatment with ozone [11]. Furthermore, no changes in color were observed after treatment of raspberries with gaseous and aqueous ozone [13, 14]. Similar results were reported for strawberries treated with gaseous or aqueous ozone [13, 14, 86]. In addition, Rocculi et al. [113] stated that strawberries washed with 1.66 ppm ozone for 5 min maintained the green color of sepals better than the control group which was washed with tap water. Reduction in firmness occurs possibly because of development of microbial activity and increase in metabolism resulting in an increase in the enzymatic activity of the produce. Since ozone exhibits biolytic effects against a wide range of microbial population, ozone-treated samples were often found to retain texture as compared to control samples. Rocculi et al. [113] did not observe a significant change in firmness in O_3-treated and untreated strawberries after a cold storage period of 20 days. On the other hand, Nadas et al. [86] stated that weight loss and softening of strawberries were reduced by exposure to 1.5 μL/L O_3-treated as compared to air-stored samples. Ozone treatments were found to insignificantly affect the firmness of some cultivars of table grapes even after 80 min of exposure [115], and even in some cases, showed the lowest losses in firmness as compared to control samples [21]. Treatment of whole and sliced apples with 3 ppm ozone dissolved in water was not reported to induce any change in the sensory quality of the product as compared to the control samples [114]. Moreover, no changes in firmness in apples and pears stored in 0.4 μL/L O_3-enriched rooms for 107 days at 20 °C were observed [121]. Favorable effects on sensory attributes as caused by ozone treatment were reported by Aguayo et al. [2]. The authors observed that tomatoes treated with gaseous ozone showed a good appearance and overall acceptability, while those stored in control conditions had sensory quality below acceptable levels. Some of the studies illustrating the effects on sensory quality of aqueous and gaseous ozone on

Table 7 Effect of gaseous ozone on some common vegetables

Type of produce	Treatment			Major findings	Reference(s)
	Concentration	Exposure time	Environment		
Broccoli	200 and 700 nL/L	12 days	12 °C	• Mold growth on the flower sepals was reduced and senescence was delayed • Chlorophyll (↓), volatile components (↓), weight loss (↑)	Forney [40]
	0.04 µL/L	7 days	10 °C 2 °C and 95–98% RH (S) (for 21 days)	• Base browning (↓), floret yellowing (↓), shelf life (↑)	Skog and Chu [121]
Bell pepper	0.7 ppm	1–5 min	–	• 2.56 LR in bacterial count • Yeasts and molds reduced to undetectable levels • Firmness (~), color (~), weight loss (~)	Horvitz and Cantalejo (2010)
Carrot	50 ± 10 µL/L	6 months	0.5 °C and 95% RH	• Reduced aerial mycelium and lesion size of *S. sclerotiorum* and *B. cinerea* • Ozone induced injury • Fresh weight loss (~), sprouting (~), glucose, fructose, galactose, sucrose (~)	Hildebrand et al. [54]
	0–5 mg/L	5 days	18 ± 2 °C and 80 ± 5% RH	• Shelf life (↑), weight loss (~), firmness (~), color (~), total soluble solids (↓)	de Souza et al. [30]
Lettuce	2.1, 5.2, and 7.6 mg/L	5, 10, 15 min	22 °C and 80% RH	• 1.79 LR for *E. coli* was observed • Color (↓)	Singh et al. [119]
Mushroom	100 mg/h	15 and 30 min	Sealed container at ambient conditions	• External browning rate (↑), internal browning (↓), color (↓), texture (~), maturity (~) weight loss (~)	Escriche et al. [33]
Onion	50 ppb during the day and 250 ppb during the night	2–4 weeks	Cold storage	• Mold incidence (↓), weight loss (↓), firmness (~), internal decay (~), sprouting (~) and rooting (~)	Song et al. [122]
Red chili peppers	0.9 µmol mol^{-1}	2 weeks	10 °C	• Disease incidence (↓), weight loss (↓), firmness (↑), skin color (↓), phenolic content (~), shelf life (↑)	Glowacz and Rees [43]
Rocket leaves	2 mg/L	8 days	–	• Bacteria and yeast count (↓), ascorbic acid (↓), phenolic content (~), total antioxidant capacity (~), PAL (~), shelf life (↑)	Gutiérrez et al. [48]
Spinach	1.6 and 4.3 mg/L	5 min	–	• 5 LR of *E. coli* O157:H7 was observed. Color (↓)	Klockow and Keener [70]
Tomatoes	0.005–1.0 µmol/mol	2–312 h	13 °C, 95% RH	• Firmness (~), soluble sugar content (~), fruit weight (~), ascorbic acid (↑), lycopene (↑), β-carotene (↑), lutein (↑), shelf life (↑)	Tzortzakis et al. [131]
	17.14 mg/m^3	1 h	0 °C, (10 ± 1) °C and (90 ± 3)% RH for 25 days (S)	• Firmness (~), ethylene (↓), volatile and aromatic compounds (~)	Liang et al. [78]]

(S)—storage conditions, *LR*—log reductions, (↑)—increase, (↓)—decrease, (~)—no change, *PAL*—phenylalanine ammonia lyase

the quality of fresh fruits are tabulated in Table 4 and Table 5, whereas those on vegetables are in Table 6 and Table 7, respectively.

Concluding Remarks

Sanitizing fresh fruits and vegetables has been one of the most important unit operations in food industries, preventing the occurrence of foodborne illnesses. Ozone, as a sanitizing agent, not only provides a sanitizing efficiency close to conventionally used chlorine, but at the same time, leaves no residues in the product. It has minimal effects on the nutritional and sensory quality of treated produce, imparting a fresh state for an extended period of storage. This paper provides evident knowledge as to how ozone can be efficiently used as a sanitizer for different fruits and vegetables, both in whole as well as minimally processed state, with primary emphasis on the factors affecting its efficiency at a technological and nutritional level. Moreover, the information highlighting the properties of ozone, factors influencing its efficacy, materials for equipment design, kinetic modeling, and its impacts on several quality parameters of fresh produce, can be useful for researchers intending to further integrate ozone as a sanitizer in the food industry. Thus, ozonation can become an integral part in the postharvest management of fruits and vegetables for providing safe and high-quality produce. In the future, research should be emphasized in economically viable automation of ozone sanitization process, with precise control on process variables assuring its effective integration in food processing line. The process variables like ozone concentration, exposure time, and other environmental factors affecting its sanitizing efficiency should be optimized to obtain maximum microbial log reductions while having a minimal effect on the product quality. Moreover, further scientific interventions are needed to ascertain the reaction kinetics of ozone when dissolved in water to predict the antimicrobial mechanism of ozone at a molecular level more clearly. Hence, the application of ozone under optimized conditions with a proper control over parameters and a minimal effect on the operator's health has the potential to replace all conventional sanitizers in the food industry in the near future for the production of safe and high-quality end products.

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