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Article *in* Food Engineering Reviews · June 2013

DOI: 10.1007/s12393-013-9064-5

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Food Eng Rev (2013) 5:77–106
DOI 10.1007/s12393-013-9064-5

REVIEW ARTICLE

A Review on Ozone-Based Treatments for Fruit and Vegetables Preservation

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Received: 4 December 2012 / Accepted: 19 February 2013 / Published online: 7 March 2013

Abstract The development and application of efficient methods for the preservation of food products have always been industrial concerns. The search for new technologies that assure safety and quality of the products is an ever-growing challenge. In this context, ozone has emerged, presenting a set of characteristics that makes it highly suitable for fruits and vegetables. Ozone properties and ozone applications to fruits and vegetables industries are included in this work. Due to the potential of ozone-based treatments for obtaining safe products with extended shelf life, several researchers have been focusing on this topic. A compilation of those works is presented in this review, with emphasis on the ozone impact on microbial inactivation and quality aspects of processed fruits and vegetables, and fruit juices as well. This review can be a helpful tool for finding process conditions of ozone-based treatments and subsequent impacts on quality and safety attributes of already studied products, opening further areas of research.

Keywords Ozone · Fruits and vegetables · Safety · Quality · Food preservation

Introduction

Fruits and vegetables are an important group of foods that represent a substantial segment of the food market. Their high consumption is related with the recognition that fresh produce are essential for a well-balanced diet, due to their nutritional value, as well as presenting color, shape, taste, aroma, and texture attractive characteristics. However, the perishable nature of these products, which determines its fast consumption and also the number of associated outbreaks, identifies the importance of applying efficient decontamination treatments.

In order to extend the shelf life of these products, conventional processes, which include the application of moderate thermal treatments, are usually applied. However, heat processing can induce several biological, physical, chemical, and microbiological changes, leading to sensorial, nutritional, and textural modifications that can negatively affect the product quality. Consequently, thermal-based treatments are not suitable for all kinds of products. Due to these drawbacks and the rising demand for fresh produce, other preservation techniques are being used. The application of several sanitizers that act as antimicrobial solutions is standard. Among them, chlorine and associated compounds are the most routinely used by the food industry [15, 80, 90]. Nevertheless, due to the formation of carcinogenic chlorinated by-products, some European countries have forbidden its use [21]. Additionally, it has been attested the inability of chlorine to effectively act on foodborne pathogens [53, 130].

The Center for Disease Control and Prevention estimates that approximately 12.3 % of all foodborne outbreaks from 1990 to 2007 were associated with the consumption of fruits and vegetables, and 21.9 % were accounted for all foodborne-associated illness [36]. When the etiologic agent was identified, 49 % of the outbreaks were caused by viruses (mainly Norovirus), 44 % were caused by bacteria (mainly *Salmonella*), 6 % by chemicals and toxins, and only 1 % were caused by parasites [30].

In light of all this knowledge, considerable research efforts have been made in the recent years to find feasible

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processing alternatives with the potential to produce fresh products with high quality and safety standards. The food industry is searching for a technology that ensures high levels of quality retention of the products, while extending their shelf life by the reduction of enzymatic activity, the destruction of pathogens and spoilage microorganisms and their toxins and by the elimination of insects, parasites, and pesticides.

From the available technologies, ozone application is a promising one, which is gaining interest in the fruit and vegetable industry. The efficacy and usefulness of ozone has been proved over the years with its widespread application in the treatment of water and food [49, 61]. In 1997, an Expert Panel of Food Scientists convened by the Electric Power Research Institute (EPRI) supported a generally recognized as safe (GRAS) classification of ozone as a disinfectant for foods when used at levels and by methods of application in accordance with good manufacturing practices. Although the Food and Drug Administration (FDA) did not objected this GRAS status, in 2000, a Food Additive Petition filled by the EPRI requested FDA approval of ozone for direct contact with foods. In 2001, FDA finally approved the application of ozone as a direct food additive for the treatment, storage, and processing of foods in gas and aqueous phases [37]. Since then, several studies had been conducted to evaluate the effectiveness of ozone in various fruits and vegetables. The ozone treatments are mainly applied in two forms: (1) the gaseous ozone can be added continuously or intermittently to an atmosphere where the produce is stored or (2) the product can be washed or dipped in water containing ozone.

The applications of ozone in the industry of fruits and vegetables have already been reviewed [8, 33, 57, 58]. Nevertheless, this work intends to collect and summarize all the studies and results obtained for fruits and vegetables treated with ozone that are not covered in previous works. It is also approached the effect of ozone on quality and safety features of the products. This review intends to compile information that will contribute to identify main achievements and what still needs to be exploited. The industrial application of ozone can be standardized to a certain product, if significant improvements in quality and safety features are attained as well as economic and competitive advantages of the technology.

Ozone Properties

Ozone is a highly unstable triatomic oxygen molecule (O_3), arranged to form an obtuse angle. It is formed by a high energy input that splits the molecular oxygen (O_2) into two O. These single O molecules rapidly combine with available O_2 to form O_3. The source of this high energy is usually electrochemical, ultraviolet radiation or electrical (corona) discharge. The first two methods have limited use due to the very high associated costs and to the poor ozone yields achieved, respectively.

The electrochemical method usually applies an electrical current, between an anode and cathode, in an electrolytic solution containing water and a solution of highly electronegative anions. This results in the production of a mixture of oxygen and ozone at the anode [72].

The ultraviolet (UV) method is based on ozone formation throughout O_2 exposure to UV radiation at a wavelength of 140–190 nm.

Corona discharge is the most commonly used method as, although it consumes large amount of electricity, it can generate the required commercial ozone levels. In this method, the feed gas (such as dry air, oxygen or a gaseous mixture) is passed between two electrodes separated by a dielectric material. For achieving higher ozone yields, oxygen must be used as the feed gas instead of air.

Once produced, ozone can be inlet directly in the gaseous form into an atmosphere or pulverized into water to produce aqueous ozone for rising and washing applications [95]. Although ozone is relatively stable in the gaseous state, it is highly unstable in aqueous solution. As it is shown in Table 1, ozone has a longer half-life in the gaseous state than dissolved in water.

In water, ozone quickly degrades to oxygen. Although ozone is extremely soluble in water (at 27 °C, ozone solubility is 580 mg/L), its solubility rate is dependent on several factors, such as pressure, temperature, pH, ozone bubble sizes, the flow rate of ozone and contact time, the purity of the water and also the technology of interchange gas/liquid [61]. The higher the temperature and pH of the solution, the lower the stability of ozone (i.e., shorter half-life). Ozone solubility increases when the purity of water increases, because the presence of minerals and organic matter in the water catalyzes ozone decomposition. Ozone solubility also increases when smaller bubble sizes are formed due to the resulting larger contact surface area.

Table 1 Typical ozone half-life based on temperature

Gaseous ozone		Aqueous ozone	
Temperature (°C)	Half-life[a]	Temperature (°C)	Half-life (min)
−50	3 months	15	30
−35	18 days	20	20
−25	8 days	25	15
20	3 days	30	12
120	1.5 h	35	8

[a] These values are based on thermal decomposition only. No wall effects, humidity, organic loading, or other catalytic effects are considered. Adapted from Goncalves [46] and OzoneSolutions [86]

Appropriate mixing or turbulence increases bubble contact and ozone solubilization [106].

At room and cold temperatures, ozone is a gas with a pungent characteristic odor, which is detectable by humans at concentrations as low as 0.02 ppm [52]. Although in low concentrations ozone is not an extremely toxic gas, at high concentrations ozone can cause severe detrimental health effects and can even be fatal. Several federal agencies have established health standards or recommendations to limit human exposure to ozone. These exposure limits are summarized in Table 2.

With an oxidation–reduction potential of 2.07 V, ozone is eligible as one of the strongest and most reactive known sanitizers. Main physical properties of ozone are summarized in Table 3. Goncalves [46] gathered information about how ozone acts and stated that three major action pathways can occur: (1) direct oxidation reactions of ozone, resulting from the action of an oxygen atom. These reactions are typically first order with high redox potential; (2) indirect oxidation reactions of ozone, where the ozone molecule decomposes to form free radicals, which will quickly react to oxidize organic and inorganic compounds; and (3) ozone may also act by ozonolysis, by fixing the complete molecule on double linked atoms, producing two simple molecules with different properties and molecular characteristics.

Application of Ozone in Fruits and Vegetables Industry

At the time of harvest, fruits and vegetables are a source of microorganisms. Without any treatment applied before consumption, they can cause serious health issues and their shelf life is reduced. Typically, fruits and vegetables are rinsed with water or chlorine solutions, which may not be sufficient to eliminate risky microorganisms that can be present. Ozone, which is environmentally friendly, has been used as an alternative sanitizer in different steps of produce processing. Indeed, ozone can even be used before harvest as it was reported by Graham et al. [47]. These authors studied the impact of aqueous ozone on tomato productivity, when it was applied via drip irrigation. Ozone is also applied industrially, in the preservation of fruit and vegetables. Apples [75] and fresh-cut salad mixes [115] are some of the products treated industrially with water containing ozone. In both cases, in addition to achieving a longer shelf life, ozonation allowed less frequent changes of flume water with subsequent lower maintenance and wastewater disposals costs. Gaseous ozone is also successfully applied to onions and potatoes for the prevention of fungal disease spread [100]. Producers, who implemented this system, were able to increase yields of marketable product enough to recover the cost of equipment investment in the first growing season of use.

Ozone has been adopted by the food industry, individually or combined with other sterilization techniques, such as ultraviolet light and ultrasounds. *Ventafresh* is a patented technology (SwissFood Tech Management, Inc.) that combines these processes. It is a method to cold-sterilize and package food, which is commercially available. The company announces that *Ventafresh* is well suited for vegetables and fruit, seafood and fish, meat and certain types of convenience food. Longer shelf life, reduced microbiological and chemical load, chlorine-free production and new products using the *Ventafresh* food processing method are disclosed. In addition, the process includes a packaging technique where food is packaged into a container and covered by a special foil, under modified atmosphere packaging techniques.

SAMRO, Ltd., a Swiss company that develops, produces and markets high-quality machines and systems for the treatment of agricultural products, has adopted *Ventafresh* technology [114]. The combination of ozone, UV radiation, and ultrasounds improves sanitation within the plant, disinfects food prior to, during, and after packaging, and thereby extends the shelf lives of products leaving the plant for distribution. The company states that this process is applicable to all root vegetables, plus root celery and asparagus, and also to certain fruits (apples, pears, kiwis, tomatoes, and Sharon fruits). In sequence, a new restaurant concept has become available, in which individually prepared (not precooked) and packaged meals are presented to the customer who eats his meal after cooked in one of many microwave ovens available [113]. All the raw foodstuffs, as wells as the packing trays and the sealing film, are disinfected using ozone.

Table 2 Reference exposure levels of ozone

Institution	Health standards
Food and Drug Administration (FDA)	Requires a concentration limit exposure of 0.05 ppm during 8 h
Occupational Safety and Health Administration (OSHA)	Requires a concentration limit exposure of 0.10 ppm during 8 h
National Institute of Occupational Safety and Health (NIOSH)	Recommends an upper limit of 0.10 ppm, not to be exceeded at any time
Environmental Protection Agency (EPA)	Requires a concentration limit exposure of 0.08 ppm during 8 h

Adapted from Goncalves [46] and OzoneSolutions [85]

Table 3 Ozone physical properties

Physical properties	Liquid phase	Gaseous phase
Molecular weight	47.98 g/mol	
Density	1,352 kg/m^3 (at −112 °C)	2.141 kg/m^3 (at 1.013 bar and 0 °C)
Boiling point	−111.3 °C (1.1013 bar)	
Melting point	−192.5 °C	
Critical point	Critical temperature: −12.2 °C Critical pressure: 55.73 bar Critical density: 540 kg/m^3	
Color	Dark blue	Pale blue

Adapted from [49] and Goncalves [46]

A different and sometimes more convenient way of fruit consumption is in the form of juice. Nevertheless, several outbreaks have been associated with this kind of product, which warns that the microbiological control of the processes cannot be neglected. The requirements are regulated by FDA, which imposes the application of treatments capable of consistently achieving at least a 5-log reduction in the level of the most resistant pathogen in the specific juice [38]. The application of ozone to fruit juices is reported to meet this FDA requirement. The impact of ozone on microbial and quality preservation of several fruit juices is presented in Table 4. Some of the research trials were conducted to validate the use of ozone in the fruit juice industry, and recently, a number of commercial fruit juice processors in the USA and Europe have started to employ ozone for juices pasteurization. As fruit juices are liquids, ozonation is frequently accomplished by ozone gas injection into the juice, usually placed in a stirred-tank reactor or bubble columns.

Effect of Ozone on Product Safety

Many contaminants (microorganisms or pesticides) are commonly associated with fresh produce. Microorganisms include bacteria (some pathogenic), viruses, parasites, and fungi/molds. The latter group is a focus of concern, not only because they cause deterioration, but also due to their potential to produce mycotoxins.

Contamination of fruits and vegetables can be related to the intrinsic microbial load of the raw material, improper handling, processing, distribution, or storage. Unless suitable measures are taken to decontaminate these products, their safety can be at risk. Behind good hygienic practices during production, processing, and transport, as well as the use of properly treated irrigation water, a measure of efficient disinfection must be applied before consumption. Typically, fruits and vegetables are rinsed with water or chlorine solutions, which may not be sufficient to eliminate all kind of microorganisms that can be present, especially in situations where the biological load is considerable high. Additionally, in some circumstances, such compounds may react with natural organic matter, forming halogenated by-products, which can cause severe health problems.

Antimicrobial efficacy of ozone has been studied for many microorganisms, including vegetative bacteria, bacterial spores, virus, yeasts, and molds. The mechanism of microbicidal action of ozone is a complex process, because ozone attacks numerous cellular constituents including proteins, unsaturated lipids and respiratory enzymes in cell membranes, peptidoglycans in cell envelopes, enzymes and nucleic acids in the cytoplasm, and proteins and peptidoglycan in spore coats and virus capsids [61].

The effectiveness of ozone treatment on microbial load depends on several factors, which may explain the diversity of published results (Tables 4, 5, 6). The main influential factors are as follows: (1) type of product; (2) target microorganism; (3) initial microbial load level; (4) physiological state of the bacterial cells; and (5) ozone physical state. The sensitivity of microorganisms to ozone is also extremely affected by the organic nature of the medium, with protection caused by physical factors, as in the case of agar, and by reduced ozone levels due to ozone demand of organic nutrients in the medium [55, 98]. Nevertheless, Restaino et al. [98] concluded that more important than the amount of organic material is the type of organic material present during ozonation.

Bacteria Inactivation

Produce contamination is mostly associated with bacteria such as *Salmonella*, *Escherichia coli*, *Listeria monocytogenes*, *Shigella*, *Campylobacter*, *Clostridium botulinum,* and *Bacillus cereus*. The majority of the studies to determine the efficacy of ozone on bacteria inactivation were carried out with *E. coli* (Tables 5, 6). This is probably related with the fact that *E. coli* O157:H7 is recognized as an important foodborne pathogen, responsible for many risky outbreaks.

Although it has been proved that ozone is effective in microbial inactivation, the sensitivity of the bacteria to ozone depends on the state in which they are. Ozone is more effective against vegetative bacterial cells than bacterial spores.

The inactivation of vegetative bacteria by ozone is achieved by the progressive oxidation of vital cellular components. It has been suggested that cell surface is the primary target of ozonation, occurring degradation of unsaturated lipids of the cell envelope. When a large part of the membrane barrier is destroyed, disruption of the cell

Table 4 Overview of the impact of gaseous ozone treatments on quality and safety characteristics of fruit juices

Fruit juice	Treatment conditions	Quality/safety characteristics	Results	References
Apple	Exposure to 33–40 ppm ozone for 8 min at 15°–18 °C and storage at 4, 8, 12, and 16 °C during 30 days A dynamic storage temperature study was also carried out.	*Saccharomyces cerevisiae* ATCC 9763	A lag phase was observed for all ozone-processed samples, probably due to the oxidizing action of the applied ozone treatment, which may exert additional stress prior to allowing growth Authors reported the effectiveness of ozone for the extension of shelf life of apple juice	Patil et al. [93]
Apple	Ozone exposure (1–4.8 % w/w) at 20 °C up to 10 min	Color Rheological properties Phenolic content	Significant changes in color, rheological properties and phenolic content were observed during ozonation	Torres et al. [122]
Apple	Exposure to 0.4 ppm ozone at 20 °C during 0–10 min	*E. coli* ATCC 25922 and NCTC 12900 Color Phenolic content	A desired 5 log reduction of *E. coli* load can be achieved within 5 min of ozone treatment Apple juice color and total phenols were significantly affected by ozone concentration and treatment time	Patil et al. [92]
Apple	Ozone exposure (12 % v/v)	Patulin (mycotoxin)	Results shown that patulin was the main target of ozone attack even in the presence of sugars, since they remained in identical levels	Cataldo [29]
Apple	Exposure to 860 ppm ozone at 20 °C for 28 min and storage at 4–6 °C for 21 days	pH Titratable acidity (TA) Turbidity Sedimentation Color °Brix Sugar content Sensorial analysis	Ozone-treated samples had greater sedimentation, lower sucrose content and a decrease in soluble solids after the 21 days of storage	Choi and Nielsen [32]
Apple	Ozone exposure (9 g/h) for (1) 90 min or (2) 60 min, followed by 24 h storage at 4 °C	*E. coli* O157:H7 *Salmonella*	Ozone treatment was more effective in reducing *Salmonella* microbial load than *E. coli*	Williams et al. [132]
Blackberry	Ozone exposure (0–7.8 %) at 20 °C up to 10 min	Anthocyanins Color	Ozone concentration and treatment time were found to be critical factors influencing both anthocyanins and color degradation	Tiwari et al. [118]
Grape	Ozone exposure (0–7.8 % w/w) at 20 °C up to 10 min	Color Anthocyanins pH °Brix Titratable acidity	No significant changes in pH, Brix and titratable acidity of ozonated samples were observed However, significant changes in the juice color and anthocyanins content were observed during ozone treatment	Tiwari et al. [119]
Orange	Exposure to 75–78 ppm ozone for different time periods (0–18 min)	*E. coli* ATCC 25922 and NCTC 12900	Generally, ozone treatment of orange juice resulted in a population reduction of 5 log-cycles	Patil et al. [91]

Table 4 continued

Fruit juice	Treatment conditions	Quality/safety characteristics	Results	References
Orange	Ozone exposure (0.6–10.0 %) at 20 °C up to 10 min	pH °Brix Titratable acidity Cloud value Non-enzymatic browning Color Ascorbic acid content	No significant changes in pH, Brix, titratable acidity, cloud value, and non-enzymatic browning were found However, ozonation was found to have a significant effect on juice color and ascorbic acid content	Tiwari et al. [116]
Orange	Ozone exposure (0.9 g/h) for 90 min and storage for 5 days at 10 °C	Ascorbic acid content Color *S. cerevisiae*	Ascorbic acid was almost fully degraded after 90 min of ozone treatment Survival of *S. cerevisiae* was not significantly different between ozonated and non-ozonated samples after storage	Angelino et al. [12]
Orange	Ozone exposure (9 g/h) for (1) 90 min or (2) 60 min followed by 24 h storage at 4 °C	*E. coli* O157:H7 *Salmonella*	Ozone treatment was more effective in reducing *Salmonella* microbial load than *E. coli*	Williams et al. [132]
Strawberry	Ozone exposure (1.6–7.8 %) at 20 °C up to 10 min	Anthocyanins Ascorbic acid Color	Significant reductions in anthocyanins (98.2 %) and ascorbic acid (85.8 %) were observed at an ozone concentration of 7.8 % w/w and a treatment time of 10 min	Tiwari et al. [120]
Tomato	Ozone exposure (1.6–7.8 %) at 20 °C up to 10 min	pH °Brix Titratable acidity Cloud value Non-enzymatic browning Color Ascorbic acid content	No significant changes in pH, Brix, titratable acidity, cloud value and non-enzymatic browning were found with ozonation Color values and ascorbic acid content changed significantly. For 10 min at the highest ozone concentration, an ascorbic acid reduction of 96 % was observed	Tiwari et al. [117]

occurs with subsequent leakage of cellular contents and bacterial cells lyses. When this is not sufficient for the immediate cell destruction, ozone can penetrate inside the bacterium and oxidize certain essential components, e.g., enzymes, proteins, and nucleic acids. This was demonstrated by Komanapalli and Lau [66], who verified that the initial target of ozone toxicity on *E. coli* cells was the bacterium membrane, affecting both lipid and protein components. Notwithstanding the membrane permeability be compromised at short time intervals of ozone exposure, the bacterial cell was still capable of surviving as there was no apparent damage of intracellular components and no change in viability occurred. However, when *E. coli* cells were subjected to prolonged ozone exposure, intracellular proteins and DNA were affected, and there was a decrease in cells viability.

Generally, Gram-negative bacteria are more sensitive to ozone than Gram-positive organisms. In Gram-negative bacteria, the lipoprotein and lipopolysaccharide layers are the prime sites of destruction, resulting in increased cell permeability and eventual cell lysis [63, 127]. The higher resistance of Gram-positive bacteria is partly explained by the greater amount of peptidoglycan in their cell walls. Rey et al. [99] showed that *N*-acetyl glucosamine, a compound present in peptidoglycan of bacterial cell walls, was resistant to the action of ozone in aqueous solutions with pH from 3 to 7.

Several researchers compared the efficiency of gaseous ozone with aqueous ozone on bacterial inactivation. Contradictory results have been reported, since some researchers verified that water containing ozone was significantly more effective [62, 96, 139] and others obtained higher microbial log reductions when gaseous ozone treatment was applied [111]. These inconsistent results are probably due to the different kind of produce used in each study. Therefore, ozone effectiveness must be assessed for

Table 5 Overview of the impact of gaseous ozone treatments on quality and safety characteristics of fruits and vegetables

Produce	Treatment conditions	Quality/safety characteristics	Results	References
Apples	Exposure to 450 ppb ozone for 48 h and storage for 12 days at 20 °C with 95–97 % relative humidity (RH)	*Botrytis cinerea* Color Chlorophyll fluorescence Texture	Lesion size was reduced on treated apples Ozone treatment had no significant effect on color or on chlorophyll fluorescence	Sharpe et al. [110]
Banana	Ozone exposure at a flow rate of 8 mL/s for 0, 10, 20, and 30 min	Total phenol and total flavonoid content Vitamin C Antioxidant activity	Results showed that ozone treatment for up to 20 min would be useful in enhancing the nutritional properties	Alothman et al. [10]
Blackberries	Storage for 12 days at 2 °C in an atmosphere with 0.0, 0.1, and 0.3 ppm of ozone	Fungal decay Anthocyanins Color POD activity	Ozone storage suppressed fungal development for 12 days, while 20 % of control fruits showed decay No impact on anthocyanin content Color was better retained in ozonated samples POD was greater in controls and samples treated with 0.1 ppm ozone	Barth et al. [16]
Blueberries	Four different gaseous treatments were applied: (1) continuous ozone exposure (50 g/kg O_3) and (2) pressurized ozone exposure (83 kPa; 50 g/kg O_3), for 2, 4, 8, 16, 32, and 64 min; (3) continuous ozone exposure followed by pressurized ozone (64 + 64 min); (4) vacuum followed by pressurized ozone for 64 min	*E. coli* O157:H7 *Salmonella* Color Sensorial analysis	Maximum reduction of *Salmonella* (3.0 log) was achieved after 64 min of pressurized ozone For *E. coli*, the maximum reduction (2.2 log) was attained after 64 min of continuous ozone exposure The combined ozone treatments, continuous followed by pressurized and vacuum followed by pressurized ozone did not result in an increase in log reductions for either *Salmonella* or *E. coli* Significant changes in color were detected, as berries appeared darker after treatment	Bialka and Demirci [22]
Cantaloupe	Ozone exposure (4.3 ppm) for 5 min and storage at room temperature for 24 h	*Salmonella poona*	Ozone gas treatment resulted in 4.29 log CFU/g reduction	Trinetta et al. [123]
Cantaloupe melon	Exposure to 5,000, 10,000 and 20,000 ppm ozone for 30 min under 90–95 % RH and 11 °C, and then storage at 5 °C for up to 7 days	*S. poona* *E. coli* *Pseudomonas fluorescens* Coliforms Yeasts/molds Lactic acid bacteria (LAB) Color Texture Soluble solids content Sensorial analysis	Ozone treatment greatly reduced initial counts of all microbial groups tested. It was effective against *Salmonella* not only initially but also during the 7 days of storage No impact of ozone treatment on visual quality, aroma, firmness and soluble solids content after treatment and storage were observed Translucency and lightness were slightly increased and decreased, respectively, after ozone treatments	Selma et al. [109]

Table 5 continued

Produce	Treatment conditions	Quality/safety characteristics	Results	References
Cantaloupe melon	Samples were exposed for 30 min to 10,000 ppm ozone (11 °C, 90–95 % RH) with (1) O_2 gas and (2) O_2 and CO_2 gas and also firstly immersed in hot water (75 °C for 1 min) and then exposed to ozone	*E. coli* O157:H7 Aerobic mesophilic bacteria (AMB) Psychrotrophic bacteria Coliforms Yeasts/molds Color Texture Soluble solids content Sensorial analysis	The combination of hot water and gaseous ozone was the most effective treatment to control microbial growth, achieving 3.8, 5.1, 2.2 and 2.3 log reductions for AMB, psychrotrophic bacteria, yeasts/molds and coliforms, respectively No significant differences were observed between gaseous ozone and gaseous ozone supplied by carbon monoxide gas No impact on quality attributes	Selma et al. [108]
Clementines	Exposure to 0.1 µmol/mol ozone and maintenance at 13 °C under 95 % RH during 13 days	*B. cinerea*	Ozone treatment suppressed spore production by as much as 95 % in comparison with 'control' fruit. Lesion development was also significantly suppressed by ozone	Tzortzakis et al. [124]
Date fruits	Exposure to ozone (1, 3, and 5 ppm) for four different periods (15, 30, 45 and 60 min)	Total bacterial counts Coliforms *Staphylococcus aureus* Yeasts/molds	A 1 h ozone treatment at 5 ppm could be successfully used for reducing coliforms and *S. aureus* of date fruits, but longer exposure times are required for elimination of the total mesophilic bacteria as well as yeasts/molds	Najafi and Khodaparast [79]
Figs (dried)	Inoculated figs were dried for 1 h at 25 °C and then exposed to gaseous ozone (0.1, 0.5 and 1.0 ppm up to 360 min for *E. coli and B. cereus*; 1.0, 5.0, 7.0 and 9.0 ppm for 360 min for *B. cereus* spores) in a chamber at 20 °C and 70 % RH	*E. coli* ATCC 8739 *B. cereus* ATCC 6633 *B. cereus* spores Color pH Moisture content Sensorial analysis	*E. coli* and *B. cereus* counts were decreased by 3.5 log numbers at 1.0 ppm ozone concentration for 360 min Up to 2 log reductions in the number of *B. cereus* spores were observed above 1.0 ppm ozone concentration at the end of 360 min of ozonation No significant changes in color, pH, moisture content, sweetness, rancidity, flavor, appearance and overall palatability of ozonated dried figs were observed	Akbas and Ozdemir [4]
Figs (dried)	Dried figs were exposed to 13.8 ppm of gaseous ozone during 7.5, 15 and 30 min for microbial studies and during 30, 60 and 180 min for aflatoxin level determination	AMB *E. coli* Coliforms Yeasts/molds Aflatoxin B1 content	Microbial counts were decreased by 0.81–1.42, 0.46–1.84. 0.16–2.09 and 0.59 log for AMB, coliforms, total yeasts and total molds, respectively *E. coli* was not detected after a 7.5 min ozone treatment Reductions of 48.77–95.21 % of aflatoxin content were achieved after the different treatment times	Zorlugenc et al. [139]
Figs (dried)	Storage for 3 and 5 h in a chamber with gaseous ozone at 5 and 10 ppm	Total bacterial counts Coliforms Yeasts/molds	Minimum 3 h treatment at 5 ppm was required to reduce microbial counts Decrease in total aerobic mesophyllic microorganisms and yeasts/molds counts was ≈38 and 72 % at 5 ppm All coliforms were inactivated	Oztekin et al. [87]
Grapes (table)	Exposure to 200, 250, or 350 ppm ozone, at 95, 75 or 35 % RH, respectively	Conidia of *Penicillium digitatum*, *Penicillium italicum* and *B. cinerea*	Conidia died more rapidly during ozone exposure at higher humidity than at lower humidity *P. digitatum* and *P. italicum* were more resistant to ozone than *B. cinerea*	Ozkan et al. [84]

Table 5 continued

Produce	Treatment conditions	Quality/safety characteristics	Results	References
Grapes (table)	Exposure to 0, 2,500, 5,000, or 10,000 ppm ozone for 1 h at 5 °C, under moderate vacuum (33 kPa) Samples exposed to 5,000 ppm were stored for 1 month at 0.5 °C	*B. cinerea* Fungicide residues	Gray mold incidence was reduced, in average, ≈50 % when samples were exposed to gaseous ozone Ozone applications reduced the content of several (fenhexamid, cyprodinil, pyrimethanil and pyraclostrobin) tested fungicides applied to control *B. cinerea*. However, iprodione and boscalid residues were not degraded by ozone	Gabler et al. [44]
Grapes (green table)	Exposure to 450 ppb ozone for 48 h and storage for 12 days at 20 °C with 95–97 % RH	*B. cinerea* Color Chlorophyll fluorescence Texture	Decay incidence of treated grapes was reduced. Ozone treatment had no significant effect on chlorophyll fluorescence	Sharpe et al. [110]
Grapes (table)	Ten different gaseous treatments, including ozone intermittent (8 ppm of O_3 for 30 min every 2.5 h) and continuous (0.1 ppm O_3 at 0 °C and 90 % RH for 1 h) applications were evaluated during 60 days of storage at 0 °C, followed by 7 days of shelf life at 15 °C in air	Color Texture Total soluble solids content pH Titratable acidity Weight loss Decay Polyphenols content Sensorial analysis	Decay was identified as being solely due to *B. cinerea* Decay values were 7.23 and 7.97 % for continuous and O_3 shock treatments, respectively against the 50 % of other treatments Generally, ozone treatments had no impact on color, firmness, total soluble solids content, pH and acidity Both O_3 treatments increased the total flavan-3-ols content (the most active antioxidant in grapes)	Artes-Hernandez et al. [13]
Grapes (table)	Exposure to 0.1 µmol/mol ozone and maintenance at 13 °C under 95 % RH during 13 days	*B. cinerea*	Ozone treatment suppressed spore production by as much as 75 % in comparison with 'control' fruit	Tzortzakis et al. [124]
Grapes (table)	Ten different gaseous treatments, including ozone intermittent (8 ppm of O_3 for 30 min every 2.5 h) and continuous (0.1 ppm O_3 at 0 °C and 90 % RH for 1 h) applications were evaluated during 38 days of storage at 0 °C followed by 6 days of shelf life at 15 °C in air	Color Texture Total soluble solids content pH Titratable acidity Weight loss Decay Phenolic compounds	Decay incidence on grapes ranged between 5.9 % for ozone shocks and 13 % for ozone continuous application Generally, ozone treatments had no impact on color, firmness, total soluble solids content, pH and acidity The major anthocyanin found in table grapes decreased in all treatments, except for grapes after shelf life subjected to ozone shocks. The same happened for the total flavonol content, for which no significant changes were found in both ozone treatments	Artes-Hernandez et al. [14]
Grapes (table)	Continuous ozone exposure at 0.3 ppm and storage for 7 weeks at 5 °C and 90 % RH	*Monilinia fructicola* *B. cinerea* *M. piriformis* *Penicillium expansum* Water loss	Gray mold nesting was nearly prevented under 0.3 ppm ozone No visible injuries on the fruit tissues were noticed during or after ozone exposure No impact on weight loss was verified	Palou et al. [88]

Table 5 continued

Produce	Treatment conditions	Quality/safety characteristics	Results	References
Grapes (table)	Ozone application (16 ppm) in a cylinder and storage at 20 °C and 85–90 % RH during 6 days	Natural microflora of fungi, yeasts and bacteria Grape decay caused by *Rhizopus stolonifer*	All the microflora naturally present on the berry surface was greatly reduced by a 20 min ozone exposure A significant decrease in decay was observed in berries that were treated with ozone either before or after being inoculated with *Rhizopus stolonifer* spores	Sarig et al. [105]
Guava	Ozone exposure at a flow rate of 8 mL/s for 0, 10, 20, and 30 min	Total phenol and total flavonoid content Vitamin C Antioxidant activity	Results showed that ozone treatment had a negative effect on the nutritional value of guava fruit	Alothman et al. [10]
Kiwifruit	Cold storage (0 °C, 95 % RH) with gaseous ozone (0.3 ppm) for 1, 3, and 5 months and then storage at 20 °C for up to 12 days	Ethylene production Respiration rate Texture Soluble solids content Titratable acidity Total antioxidant activity Ascorbic acid and phenolic content	Ozone blocked ethylene production, delayed ripening, and stimulated antioxidant and antiradical activities of kiwifruit Results also suggested that ozone is efficient in promoting higher firmness retention Overall, data indicate that ozone improved kiwifruit post-harvest behavior	Minas et al. [76]
Lemons	Exposure to an intermittent day-night ozone cycle (0.3 ppm) at 4.5 °C for 9 weeks and then continuous ozone exposure (1.0 ppm) at 10 °C for 2 weeks	*P. digitatum* *P. italicum* Development of postharvest green and blue molds	The incidence of blue mold was delayed, but not reduced, by a day-night ozone cycle. In contrast, the sporulation of blue mold was greatly reduced by a day-night ozone cycle Green mold incidence was significantly lower among fruit exposed to 1.0 ppm ozone storage	Palou et al. [89]
Longan fruit	Ozone exposure (200 µL/L) alone or combined with citric, ascorbic or oxalic acid for 0, 15, 30, 60 and 120 min and then storage at 25 °C	Natural microflora of fungi, yeasts and bacteria Browning Polyphenol oxidase (PPO) activity	Exposing fruits to ozone for 60 and 120 min significantly reduced microbial population on fruit surface Fruit treated with ozone in combination with oxalic or citric acid had less browning and a reduction of PPO activity	Whangchai et al. [131]
Oranges	Continuous exposure to 0.3 ppm ozone at 5 °C for 4 weeks, and then ozone continuous exposure (1.0 ppm) at 10 °C for 2 weeks	*P. digitatum* *P. italicum* Development of postharvest green and blue molds	Growth of *P. italicum*, but not of *P. digitatum*, was significantly reduced by a 0.3 ppm ozone exposure	Palou et al. [89]
Peaches	Continuous ozone exposure at 0.3 ppm, and storage for 4 weeks at 5 °C and 90 % RH	*Monilinia fructicola* *B. cinerea* *Mucor piriformis* *P. expansum* Respiration and ethylene production rates Water loss	Inhibition of aerial mycelial growth and sporulation was verified No impact on respiration and ethylene production rates was observed Continuous ozone exposure increased water loss	Palou et al. [88]
Pineapple	Ozone exposure at a flow rate of 8 mL/s for 0, 10, 20, and 30 min	Total phenol and total flavonoid content Vitamin C Antioxidant activity	Results showed that ozone treatment for up to 20 min would be useful in enhancing the nutritional properties	Alothman et al. [10]

Table 5 continued

Produce	Treatment conditions	Quality/safety characteristics	Results	References
Plums	Exposure to 0.1 µmol/mol ozone and maintenance at 13 °C under 95 % RH during 13 days	*B. cinerea*	Ozone treatment suppressed spore production by as much as 20 % in comparison with 'control' fruit Ozone treatment resulted in no effects on lesion development	Tzortzakis et al. [124]
Raspberries	Four different gaseous treatments were applied: (1) continuous ozone exposure (50 g/kg O_3) and (2) pressurized ozone exposure (83 kPa; 50 g/kg O_3), for 2, 4, 8, 16, 32, and 64 min; (3) continuous ozone exposure followed by pressurized ozone (64 + 64 min); (4) vacuum followed by pressurized ozone for 64 min	*E. coli* O157:H7 *Salmonella entérica*	Maximum reductions were achieved with treatment (3) 3.55 and 3.75 log reductions were achieved for *Salmonella* and *E. coli*, respectively	Bialka and Demirci [24]
Strawberries	Exposure to 40 ppm ozone for 1, 5, 30, 60, or 120 min	Biothiol content	At 40 ppm, ozone treatment for 30 min was found to be optimal for disinfection purposes, maintaining beneficial biothiols levels	Demirkol et al. [35]
Strawberries	Four different gaseous treatments were applied: (1) continuous ozone exposure (50 g/kg O_3) and (2) pressurized ozone exposure (83 kPa; 50 g/kg O_3), for 2, 4, 8, 16, 32, and 64 min; (3) continuous ozone exposure followed by pressurized ozone (64 + 64 min); (4) vacuum followed by pressurized ozone for 64 min	*E. coli* O157:H7 *S. entérica*	Maximum reductions were achieved with treatment (3) 2.60 and 2.96 log reductions were achieved for *Salmonella* and *E. coli*, respectively	Bialka and Demirci [23]
Strawberries	Storage for 3 days at 2 °C in an atmosphere with 1.5 µL/L ozone and transferred to room temperature	*B. cinerea* Fungal decay Weight loss Texture Aroma	Visible mycelial growth developed more slowly on strawberries previously stored in ozone-enriched cold atmosphere Decay incidence, weight loss, and fruit softening were reduced in samples stored in ozone atmosphere. However, a reversible loss of fruit aroma was obtained	Nadas et al. [78]
Strawberries	Storage for 3 days at 2 °C in an atmosphere with 0.35 ppm ozone and then storage for 4 days at 20 °C	Fungal decay Total anthocyanin content Sugar and acids distribution Aroma	Ozone treatment was partially effective in preventing fungal growth after 2 days at 20 °C, with 15 % less fungal decay. However, it was ineffective in preventing fungal decay after 4 days at 20 °C After 3 days at 2 °C, treated samples showed a significant decrease in anthocyanin content, significant differences in sugars and 3 times higher values of ascorbic acid A detrimental effect of ozone treatment on strawberry aroma was also observed	Perez et al. [94]
Tangerine	Ozone exposure (200 ppm) for 0, 2, 4 or 6 h and storage for 3 days at 25 °C with 75–80 % RH	*P. digitatum* Activity of antioxidant enzymes (superoxide dismutase, catalase and ascorbate peroxidase)	Ozone treatment for 4 and 6 h reduced fungi growth The activities of antioxidant enzymes increased after ozone fumigation and remained significantly higher through storage	Boonkorn et al. [28]

Table 5 continued

Produce	Treatment conditions	Quality/safety characteristics	Results	References
Tomatoes	Ozone exposure (4.3 ppm) for 5 min and storage at room temperature for 24 h	*S. poona*	Ozone gas treatments resulted in 4.06 log CFU/g reduction	Trinetta et al. [123]
Tomatoes	Exposure to 10 ppm ozone for 5, 10, or 20 min, and storage for 9 days at 20 °C with 90 % RH	Color pH Sugars content Antioxidant activity Texture Weight loss Respiration rate Pectin and hemicelluloses solubilization	Ozone treatments had no impact on fruit color, sugar content, acidity, or antioxidant capacity, but reduced fruit damage and weight loss and induced the accumulation of phenolic compounds Softening was delayed in ozone-treated fruit Cell wall analysis showed that exposure to ozone decreased pectin but not hemicelluloses solubilization	Rodoni et al. [103]
Tomatoes	Exposure to 25,000 and 45,000 ppm for 2 h/day during 16 days, under not controlled temperature and RH (mean values of 27 °C and 60 % RH)	AMB Soluble solids content Texture pH Lycopene content Vitamin C	Results showed that ozone exposure during storage extends the shelf life of tomatoes, besides preserving its sensorial attributes (No impact on soluble solids content or on Vitamin C were observed; smaller weight loss and higher firmness was detected in ozonated samples)	Venta et al. [126]
Tomatoes	Exposure to 20, 35 and 50 ppm ozone for 10 min, and storage at 15, 25 and 35 °C with 68 % RH	Color	Ozone treatment delayed both the development of red color as well as of rotting Shelf life was enhanced by 12 days when treated tomatoes were stored at 15 °C, mainly due to a reduction in surface microbial count	Zambre et al. [137]
Tomatoes	Exposure to 0.05, 0.2, 1.0 or 5.0 ppm ozone up to 312 h, under dark at 13 °C, 95 % RH, and then storage for up to 13 days	*Alternaria alternata* *Colletotrichum coccodes*	Low level of ozone resulted in a modest, but statistically significant, reduction in fungal lesion development Higher concentrations of the gas resulted in greater effects	Tzortzakis et al. [125]
Tomatoes	Exposure to 0.05–5.0 µmol/mol ozone, and maintenance at 13 °C under 95 % RH during 13 days	*B. cinerea*	Ozone treatment suppressed spore production by as much as 50 % in comparison with 'control' fruit Lesion development was significantly suppressed by ozone	Tzortzakis et al. [124]
Tomatoes (whole and sliced)	Storage for 15 days at 5 °C and 95 % RH in an atmosphere enriched to 4 µL/L ozone during 30 min each 3 h	Respiration and ethylene production rates Color Texture Soluble solids content Sensorial analysis AMB Psychrotrophic bacteria Yeasts/molds	Results showed a decrease of respiration rate and metabolic activity and an increase of sugar and organic acids content Although tissue firmness was maintained and the ozone-treated fruit retained a good appearance and overall quality, aroma decayed Ozone substantially reduced microbial counts, being more noticeable on bacteria (1.1–1.2 log) than on fungi (0.5 log)	Aguayo et al. [2]

Table 5 continued

Produce	Treatment conditions	Quality/safety characteristics	Results	References
Tomatoes (cherry)	Exposure to 5, 10, 20 and 30 ppm ozone for 5, 10, 15 and 20 min and then packaging in modified atmosphere and storage at 7 and 22 °C	*S. enteritidis* PT4 E10 LAB Color Texture	Complete reduction of *S. enteritidis* at 20 ppm after 15 min treatment. *S. enteritidis* can survive storage, depending on the location site of the pathogen on the fruit, the load of contamination and the storage temperature Color changes for the higher ozone concentrations with texture maintenance	Das et al. [34]
Asparagus	Exposure to 40 ppm ozone for 1 h	Biothiol content Total bacteria counts	Ozone exerted minimal effect on biothiol concentrations 0.75 log reductions were observed	Qiang et al. [96]
Broccoli	Broccoli treated with 1-methylcyclopropene was stored in an atmosphere with 0, 200 and 700 nL/L ozone at 12 °C with 95–97 % RH for up to 12 days	Color Weigh loss Respiration and ethylene rates Chlorophyll fluorescence Decay Volatile compounds	Application of 200 nL/L ozone showed no significant impact on color, weight loss, respiration and ethylene production and chlorophyll fluorescence, while 700 nL/L ozone treatments were injurious Both ozone concentrations caused reduction or elimination of decay	Forney et al. [40]
Carrots	Exposure to 450 ppb ozone for 48 h, and storage for 12 days at 20 °C with 95–97 % RH	*B. cinerea* *Sclerotinia sclerotiorum* Color Chlorophyll fluorescence Texture	Lesion size and height of the aerial mycelium were significantly reduced by the ozone treatment Ozone treatment had no significant effect on color	Sharpe et al. [110]
Carrots	Carrots treated with 1-methylcyclopropene were exposed to an atmosphere with 0, 300 and 1,000 nL/L ozone at 10 °C for 0, 1, 2 or 4 days, and stored at 0 °C for up to 24 weeks	*B. cinerea* *S. sclerotiorum* Respiration and ethylene rates Texture Sugars Volatile compounds	Ozone treatments (1,000 nL/L) induced decay resistance to *B. cinerea*, but not to *S. sclerotiorum*. However, this treatment reduced carrots quality through the increase of respiration rates and the reduction of firmness and sucrose content Ozone treatments also stimulated the production of stress volatiles compounds	Forney et al. [41]
Carrots (baby)	Exposure to 2.1, 5.2, and 7.6 ppm ozone for 5, 10, and 15 min, under 80 % RH and 22 °C, respectively	*E. coli* O157:H7	Ozone treatments inactivated *E. coli* by 1.11–2.64 log CFU/g The bactericidal effect increases with concentration, and length of exposure to gaseous ozone	Singh et al. [111]
Carrots	Intermittent flush of ozone (0, 7.5, 15, 30, or 60 mL/L) into chambers for 8 h daily during 28 days at storage temperatures of 2, 8 and 16 °C	*B. cinérea* *S. sclerotiorum* Weight loss Color	Results suggested that an ozone supply of 15 mL/L for 8 h a day at 2 °C could provide some disease protection with a minimum of physical and physiological damage	Liew and Prange [70]
Cucumber	Exposure to 40 ppm ozone for 1 h	Biothiol content Total bacteria counts	1.04 log reductions on bacterial counts were observed	Qiang et al. [96]
Green bean	Exposure to 40 ppm ozone for 1 h	Biothiol content Total bacteria counts	Ozone did not decrease the level of biothiols 0.38 log reductions were observed	Qiang et al. [96]
Lettuce	Ozone exposure (4.3 ppm) for 5 min, and storage at room temperature for 24 h	*E. coli* O157:H7	Ozone gas treatments resulted in 3.53 log CFU/g reduction	Trinetta et al. [123]

Table 5 continued

Produce	Treatment conditions	Quality/safety characteristics	Results	References
Lettuce	Exposure to 2.1, 5.2, and 7.6 ppm ozone for 5, 10, and 15 min, under 80 % RH and 22 °C, respectively	*E. coli* O157:H7	Ozone treatments inactivated *E. coli* by 0.79–1.79 log CFU/g The bactericidal effect increases with concentration, and length of exposure to gaseous ozone	Singh et al. [111]
Lettuce	Ozone flush into a chamber with and without prior application of vacuum	Microbial counts	When vacuum was applied before the ozone flush, a higher decrease in the total microbial counts was achieved	Kim et al. [62]
Pepper (green)	Samples were treated with 2–8 ppm for 10–40 min, under 60–90 % RH and at 22 °C	*E. coli* O157:H7	Ozone gas concentration, RH and time were all significant factors for the inactivation of *E. coli*, being ozone concentration the most important	Han et al. [50]
Pepper (red)	Exposure to 40 ppm ozone for 1 h	Biothiol content Total bacteria counts	On average, ozone decreases the mean level of biothiols by approximately 40 % 1.0 log reductions were observed	Qiang et al. [96]
Pumpkin	Exposure to intermittent ozone (150 ppb, 5 days, 5 h/day) in a fumigation chamber	Ascorbate peroxidase activity Vitamin C Phenols content	Results support the hypothesis that ozone stimulates the antioxidant systems and that ascorbic peroxidase activity, ascorbic acid levels and cell wall stiffening are the most influenced parameters	Ranieri et al. [97]
Spinach	Prepackaged samples were exposed to ozone (1.6 and 4.3 ppm) during 5 min, and then stored at 5 and 22 °C for 0.5, 2 and 24 h in air or oxygen atmosphere	*E. coli* O157:H7 Color Relative humidity	All treated samples showed reductions in *E. coli* populations with the largest reductions (3–5 log CFU/leaf) after 24 h of storage Treated spinach showed discoloration Only leaves stored or treated in oxygen atmosphere showed relative humidity changes, indicating that ozone generation in oxygen (and not in air) has an effect on relative humidity levels inside the package	Klockow and Keener [65]
Spinach	Treatment in a pilot-scale system with combinations of vacuum cooling (4 °C) and high gaseous ozone levels (935 ppm) for 30 min A separate treatment used low ozone levels (5–10 ppm) during 3 days The combination of the two treatments was also studied	*E. coli* O157:H7	The combination of vacuum cooling with high gaseous ozone levels decreased *E. coli* load by 1.8 log CFU/g with no apparent damage to the quality of spinach Refrigerated samples treated with low ozone levels for 3 days, in a system that simulated sanitization during transportation, decrease *E. coli* load by 1.4 log CFU/g When the treatments were applied sequential, *E. coli* was undetectable (i.e., >5 log reduction) after the 3 days of process	Vurma et al. [128]
Spinach	Exposure to 40 ppm ozone for 1 h	Biothiol content Total bacteria counts	On average, ozone decreases the mean level of biothiols by approximately 53 % 0.28 log reductions were observed	Qiang et al. [96]

Table 6 Overview of the impact of aqueous ozone treatments on quality and safety characteristics of fruits and vegetables

Produce	Treatment conditions	Quality/safety characteristics	Results	References
Apples	Immersion in water containing ozone (3 ppm) for up to 5 min, and then storage at 4 °C for 9 days	*E. coli* O157:H7 *L. monocytogenes* AMB Yeasts/molds	Ozone was effective in the inactivation of *E. coli* and *L. monocytogenes* Populations of both pathogens remained relatively unchanged, whereas numbers of AMB and yeasts/molds increased during storage	Rodgers et al. [102]
Apples	Ozone bubbling during apple washing and dipping apples in pre-ozonated water	*E. coli* O157:H7	Bubbling was more effective than dipping (*E. coli* counts decreased 3.7 and 2.6 log CFU, respectively on apple surface) Optimum conditions for decontamination of whole apples with ozone: pretreatment with a wetting agent, followed by bubbling ozone for 3 min (↓ 3.3 log CFU/g)	Achen and Yousef [1]
Apples	Treatments of whole apples with ozonated wash water in a bench top prototype chamber designed by the authors	*E. coli* O157:H7	A maximum reduction of 2.9 log CFU in *E. coli* was yielded in apples surface	Klingman and Christy [64]
Apples	Washing in water containing ozone (0.25 ppm) for 30 min	Pesticides	Dipping in water containing ozone resulted in reduced levels of pesticides on apples surface	Ong et al. [83]
Blueberries	Washing in water containing ozone (1.7–8.9 ppm) at 20 °C for 2, 4, 8, 16, 32, and 64 min Washing in water containing ozone (21 ppm) at 4 °C for 64 min	*E. coli* O157:H7 *Salmonella* Color Sensorial analysis	At 20 °C, maximum reduction of *Salmonella* and *E. coli* was 4.9 log CFU/g for 32 and 64 min, respectively When the temperature was decreased to 4 C, the log reductions increased to 5.2 and 6.2 log CFU/g for *E. coli* and *Salmonella*, respectively	Bialka and Demirci [22]
Cantaloupe	Immersion in water containing ozone (3 ppm) for up to 5 min and then storage at 4 °C for 9 days	*E. coli* O157:H7 *L. monocytogenes* AMB Yeasts/molds	Ozone was effective in the inactivation of *E. coli* and *L. monocytogenes* Populations of both pathogens remained relatively unchanged, whereas numbers of AMB and yeasts/molds increased during storage	Rodgers et al. [102]
Citrus fruits (lemons, grapefruit and oranges)	Fruits were placed in baskets and immersed in water containing ozone at 4.0–10 ppm	Green mold and sour rot caused by *P. digitatum* and *Geotrichum citri-aurantii*	Green mold and sour rot were not reduced by treatments in water containing ozone	Smilanick et al. [112]
Figs (dried)	Exposure to water containing ozone (1.7 ppm), by ozone gas bubbling during 7.5, 15 and 30 min for microbial studies and during 30, 60 and 180 min, for aflatoxin level determination	AMB *E. coli* Coliforms Yeasts/molds Aflatoxin B1 content	Microbial counts were decreased by 1.49–2.42, 1.33 and 1.73 log. for AMB, total yeasts and molds, respectively *E. coli* and coliforms were not detected after a 7.5 min ozone treatment Reductions of 0.76–88.62 % of aflatoxin content were achieved after the different treatment times	Zorlugenc et al. [139]
Grapes (table)	Fruits were placed in baskets, immersed in water containing ozone at 10 ppm for 1–6 min	Gray mold caused by spores of *B. cinerea*	Immersion of table grapes for 1 min in water containing ozone at 10 ppm reduced gray mold from 35 to 10 %	Smilanick et al. [112]
Melon	Water containing ozone (4.0 or 8.0 ppm) was sprayed twice everyday (10 a.m. and 2 p.m.) for 3 successive days, under well-ventilated conditions	Visible injury	Results indicated that intensive water containing ozone spraying with a high concentration, for airborne-disease control, does not cause any visible injury to the seedlings used, as long as the spraying is carried out under well-ventilated conditions	Fujiwara et al. [43]
Nectarines	Fruits were placed in baskets, immersed in water containing ozone at 5 ppm for up to 5 min and stored for 7 days at 15 °C	Brown rot caused by *Monilinia fruticola*	Nectarines were injured only by the most severe ozone treatments	Smilanick et al. [112]

Table 6 continued

Produce	Treatment conditions	Quality/safety characteristics	Results	References
Papaya	Dipping in water containing 4 ppm of ozone for 1 or 2 min and storage at 25 °C for 10 days	Cuticular surface changes	Results shown that ozone applications did not affect the fruit's cuticular surface	Kechinski et al. [59]
Peaches	Fruits were placed in baskets, immersed in water containing ozone at 5 ppm for up to 5 min, and stored for 7 days at 15 °C	Brown rot caused by *Monilinia fruticola* Natural aerobic bacteria	Incidence of brown rot in peaches was reduced from 10.9 to 5.4 % by 1 min immersion in water containing ozone at 1.5 ppm Immersion for 1 or 5 min in water containing ozone at 5 ppm reduced aerobic bacteria populations by 1.1 and 1.6 log units, respectively	Smilanick et al. [112]
Raspberries	Washing in water containing ozone (1) 1.7–8.9 ppm at 20 °C for 2–64 min, and (2) 21 ppm at 4 °C for 64 min	*E. coli* O157:H7 *S. enterica*	Maximum reductions were 5.6 and 4.5 log CFU/g for *E. coli* and *Salmonella*, respectively, at 4 °C	Bialka and Demirci [23]
Strawberries	Dipping in water containing ozone at 0.3 ppm for 2 min, and storage for 12 days at room temperature (15 °C) or under refrigerated conditions (4 °C)	Total mesophiles Yeasts/molds Color Texture pH Total anthocyanins Vitamin C	Ozone application before storage was efficient in controlling growth of microbial contamination and attained a product with satisfactory quality retention The impact of ozone was greater when samples were maintained at room temperature	Alexandre et al. [6]
Strawberries	Dipping in water containing ozone at 0.3 and 2.0 ppm for 1, 2 and 3 min	Total mesophiles	The highest microbial reductions were obtained for the highest concentration during 3 min (2.3 log-cycles reduction)	Alexandre et al. [9]
Strawberries	Dipping in water containing ozone at 0.3 ppm for 1, 2 and 3 min	Total mesophiles	Ozone added an additional reduction of more than 0.4 log-cycles in comparison with water washings. This difference was higher particularly for short contact times	Alexandre et al. [7]
Strawberries	Dipping in water containing ozone (8.0 ppm) for 1, 5, 30, 60, or 120 min	Biothiol content	Ozone treatment for 30 min was found to be optimal for disinfection purposes, maintaining beneficial biothiols levels	Demirkol et al. [35]
Strawberries	Washing in water containing ozone (1) 1.7–8.9 ppm at 20 °C for 2–64 min, and (2) 21 ppm at 4 °C for 64 min	*E. coli* O157:H7 *S. enterica*	Maximum reductions were 2.9 and 3.3 log CFU/g for *E. coli* and *Salmonella*, respectively, at 20 °C	Bialka and Demirci [23]
Strawberries	Samples were exposed to water containing ozone (1, 3, 6 and 10 ppm) for 5 and 10 min in a batch ozone reactor designed by the authors, and stored for 21 days	Mesophilic bacteria Psycrotrophic bacteria Yeasts/molds Respiration rate Texture	Ozone caused 1.28, 1.51 and 0.78 log reductions on mesophiles, psychotrophes and yeasts/molds, respectively Little impact on firmness over storage was noted No color changes were observed after ozone treatment	Wei et al. [130]
Strawberries	Immersion in water containing ozone (3 ppm) for up to 5 min, and then storage at 4 °C for 9 days	*E. coli* O157:H7 *L. monocytogenes* AMB Yeasts/molds	Ozone was effective in the inactivation of *E. coli* and *L. monocytogenes* Populations of both pathogens remained relatively unchanged, whereas numbers of AMB and yeasts/molds increased during storage	Rodgers et al. [102]
Tomatoes	Water containing ozone (4.0 or 8.0 ppm) was sprayed twice everyday (10 a.m. and 2 p.m.) for 3 successive days, under well-ventilated conditions	Visible injury	No yellowing, chlorosis, necrosis, or malformation was observed during storage	Fujiwara et al. [43]
Tomatoes	Washing in water containing ozone (1) 0.05–0.1 ppm and (2) 0.1–0.2 ppm	AMB *S. enterica* *E. coli*	Ozone washings significantly reduced AMB counts, but did not significantly reduced *E. coli* or *Salmonella*	Long et al. [71]

Table 6 continued

Produce	Treatment conditions	Quality/safety characteristics	Results	References
Tomatoes	Washing in water containing ozone at 0.5 and 1.0 ppm during 15 and 30 min in reactors with proper agitation	*E. coli* ATCC 25922	Aqueous ozone disinfection effectiveness was observed Authors suggest that to achieve an adequate disinfection, 1 ppm ozone during 15 min must be applied	Venta et al. [126]
Tomatoes	Washing with water containing ozone (1.5–3.8 ppm) for 10 min	Spores of *Mucor piriformis*, *B. cinerea* and *Phytophthora parasitica*	Spores of *B. cinerea* on the surface of non-injured tomato were inactivated. However, when spores were placed in injured tomato surface, they were not inactivated	Ogawa et al. [81]
Watermelon	Water containing ozone (4.0 or 8.0 ppm) was sprayed twice everyday (10 a.m. and 2 p.m.) for 3 successive days, under well-ventilated conditions	Visible injury	Results indicated that intensive water containing ozone spraying with a high concentration, for airborne-disease control, does not cause any visible injury to the seedlings used, as long as the spraying is carried out under well-ventilated conditions	Fujiwara et al. [43]
Asparagus (green)	Dipping in water containing ozone (1 ppm) for 30 min and storage in modified atmosphere packaging (MAP) at 3 °C for 25 days	Lignifying changes Antioxidant enzyme activities Cell wall compositions changes	The enzyme activities including phenylalanine ammonia-lyase (PAL), superoxide dismutase (SOD), ascorbate peroxidase (APX) and glutathione reductase (GR) were inhibited by aqueous ozone treatment An increase in lignin, cellulose and hemicelluloses contents of cell wall was observed	An et al. [11]
Asparagus	Dipping in water containing ozone (8.0 ppm) for 30 min	Biothiol content Total bacteria counts	Ozone exerted minimal effect on biothiol concentrations 0.85 log reductions were observed	Qiang et al. [96]
Carrots	Dipping in water containing ozone (10 ppm) for 10 min and storage in air or in Modified Atmosphere Packaging (MAP)	Respiration and ethylene rates Ascorbic acid content Total phenolics Total carotenoid Lignin content Activity of oxidative enzymes (PPO and POD) Color AMB Coliforms Yeasts/molds Sensorial analysis	Ozonation reduced lignifications, maintaining the quality of carrots during MAP storage Maximum decrease in respiration and ethylene emission rates were obtained by the combination of ozone with MAP storage Significant reductions in ascorbic acid, carotenoids and oxidative enzymes were observed due to ozonation and MAP storage Ozone reduced microbial population and MAP storage further restricted microbial growth during storage	Chauhan et al. [31]
Carrots	Carrots were placed in baskets and immersed in water containing ozone at 1 ppm for 5 min at 5 °C	Microbial load reductions Soluble solids content pH Sensorial attributes	Water containing ozone promoted a microbial reduction up to 0.4 log for AMB and slightly higher values for yeasts/molds (0.6–0.7 log reductions) Losses of soluble solid content, color changes and impairment of aroma perception were observed	Alegria et al. [5]
Carrots (baby)	Washing with water containing ozone at 5.2, 9.7, and 16.5 ppm for 1, 5, 10, and 15 min Combinations of water containing ozone (9.7 ppm for 10 min), aqueous chlorine dioxide (10 ppm for 10 min) and thyme oil (1.0 ppm for 5 min)	*E. coli* O157:H7	*E. coli* populations were significantly reduced after 10 min washing in treatments with water containing ozone at 9.7 or 16.5 mg/L Sequential washing were very effective in killing *E. coli*, especially in the following order: thyme oil/aqueous ClO_2/water containing ozone	Singh et al. [111]

Table 6 continued

Produce	Treatment conditions	Quality/safety characteristics	Results	References
Celery	Dipping into water containing ozone at 0.03, 0.08 and 0.18 ppm for 5 min and storage for 9 days at 4 °C	Total bacterial counts PPO activity Respiration rate Vitamin C Total sugar content Sensorial analysis	Water containing ozone reduced the microbial population, being the best treatment the one with 0.18 ppm PPO activity and respiration rate were inhibited by ozone treatment, and sensorial quality of treated celery was better Ozone had no impact on Vitamin C and total sugar content	Zhang et al. [138]
Cilantro (chinese parsley)	5 min washing in water containing ozone and in water containing ozone followed by an acidic electrolyzed water (AEW) wash, packaging in a modified atmosphere and storage at 0 °C for 14 days	Total Aerobic plate counts *Enterobacteriaceae* Color Texture Sensorial analysis	Sequential wash (aqueous ozone followed by AEW) is effective in reducing initial total aerobic plate count and maintaining a relatively low microbial count during storage However, the combination of ozone and AEW led to more tissue injury, which influences the overall quality of cilantro	Wang et al. [129]
Cucumber	Water containing ozone (4.0 or 8.0 ppm) was sprayed twice everyday (10 a.m. and 2 p.m.) for 3 successive days, under well-ventilated conditions	Visible injury	No yellowing, chlorosis, necrosis, or malformation was observed during storage	Fujiwara et al. [43]
Cucumber	Dipping in water containing ozone (8.0 ppm) for 30 min	Biothiol content Total bacteria counts	2.44 log reductions on bacterial counts were observed	Qiang et al. [96]
Green bean	Dipping in water containing ozone (8.0 ppm) for 30 min	Biothiol content Total bacteria counts	Ozone exerted minimal effect on biothiol concentrations 0.81 log reductions were observed	Qiang et al. [96]
Lettuce	Pre-treatments studies (0.5–4.5 ppm for 0.5–3.5 min) Washing with water containing ozone (2 ppm) and storage at 4 °C for 12 days	*L. monocytogenes* AMB *Enterobactericeae* Psychrotrophic bacteria Vitamin C β-carotene Sensorial analysis	Water containing ozone at 2 ppm for 2 min was found to be the optimum processing conditions for lettuce disinfection AMB, psychrotrophic counts and *Enterobacteriaceae* decreased by about 1.5, 1.1 and 1.5 log CFU/g, respectively Ozonated samples presented Vitamin C and β-carotene retention	Olmez and Akbas [82]
Lettuce	Dipping in water containing ozone (4 ppm), and storage at 4 °C for 12 days	Mesophilic bacteria Psychrotrophic bacteria Color Vitamin C β-carotene Texture Moisture content	Water containing ozone reduced the populations of mesophilic and psychrotrophic bacteria by 1.7 and 1.5 log CFU/g, respectively Dipping lettuce in water containing ozone did not significantly affect the gas composition of packages, color, texture, moisture content or β-carotene and vitamin C values	Akbas and Olmez [3]
Lettuce	Washing in water containing ozone at the following conditions: (1) 1 ppm for 3 min; (2) 2 ppm for 5 min; and (3) 5 ppm for 5 min	*Shigella sonnei*	*S. sonnei* counts were reduced by 0.7, 0.9 and 1.8 log units when exposed to condition (1), (2) and (3), respectively	Selma et al. [107]
Lettuce	Samples were exposed to water containing ozone (1, 3, 6 and 10 ppm) for 3, 5 and 10 min in a batch ozone reactor designed by the authors, and stored for 21 days	Mesophilic bacteria Psycrotrophic bacteria Yeasts/molds Respiration rate Texture	Ozone caused 0.79, 1.17 and 0.99 log reductions on mesophiles, psychotrophes and yeasts/molds, respectively Little impact on firmness over storage Ozone treatment caused an increase on browning	Wei et al. [130]

Table 6 continued

Produce	Treatment conditions	Quality/safety characteristics	Results	References
Lettuce	Washing with water containing ozone (3, 5, and 10 ppm) for 5 min at ambient temperature or washing in hot water (50 °C, 2.5 min) followed by water containing ozone (5 ppm, 2.5 min) and storage at 10 °C for 6 days	AMB PAL activity Color Vitamin C Browning	There was no further bacterial reduction (1.4 log CFU/g) above 5 ppm ozone Browning increased dramatically in lettuce treated with water containing ozone at 10 ppm The combined treatment of hot water followed by water containing ozone had the same bactericidal effect as treatment with aqueous ozone alone Ascorbic acid content was not affected	Koseki and Isobe [67]
Lettuce	Lettuce samples were placed in baskets and (1) immersed in water containing ozone at 1.0 ppm for 1 min and dried for 5 min, and (2) immersed in water containing ozone (1.0 ppm, 1 min), dried for 5 min and immersed in calcium lactate (15 g/L) at 50 °C Both samples were packaged and stored for 10 days at 4 °C	Respiration rate PPO and POD activity PME Browning Texture Color Sensorial analysis	Higher respiration rates were observed in samples treated with ozone than with combined treatments Ozone showed higher efficacy in the reduction of PPO and POD Pectin methylesterase activity was reduced with ozone, showing a negative effect on textural properties Samples subjected to the double treatment showed greater changes in color than samples subjected to ozone alone At the end of storage, no appearance differences were observed between samples washed with ozone alone or combined with CLac	Rico et al. [101]
Lettuce	Dipping in water containing ozone (1, 3, and 5 ppm) alone at different exposure times (0.5, 1, 3, or 5 min), and combinations of 3 ppm ozone with 1 % organic acids (acetic, citric, or lactic acids) for 1 min exposure and storage for 10 days at 15 °C	*E. coli* O157:H7 *L. monocytogenes*	Results showed that 5 ppm ozone treatment for 5 min gave 1.09 and 0.94 log reductions of *E. coli* and *L. monocytogenes*, respectively Immersion treatment for 1 min with 3 ppm ozone combined with 1 % citric acid, resulted in 2.31 and 1.84 log reductions on *E. coli* and *L. monocytogenes*, respectively	Yuk et al. [136]
Lettuce	Washing with water containing ozone at 10, 20 and 10 ppm activated by ultraviolet C and storage in modified atmosphere for 13 days at 4 °C	Mesophilic bacteria Psycrotrophic bacteria Coliforms Respiration rate Sensorial analysis Phenolic compounds Vitamin C	The most efficient treatments were ozone at 20 ppm and ozone 10 ppm activated by UV-C. 1.6 and 3.2 log reduction on aerobic mesophilic and coliforms counts were achieved, respectively Treatments with water containing ozone resulted in good retention of sensorial quality and browning control with no detrimental reduction in the antioxidant constituents	Beltran et al. [19]
Lettuce	Prewashing in water containing ozone (1 ppm, 4 °C, 120 s) or chlorine (100–200 ppm) and subsequent washing in tap water (4 °C, 90 s), and storage at 4 °C for up to 9 days	PAL, PPO and POD activity Caffeic acid derivatives content	Compared to tap water and water containing ozone, the use of chlorinated water significantly reduced PAL activity and the associated rise of 3,5-di-O-caffeoylquinic acid concentrations	Baur et al. [18]
Lettuce	Prewashing in water containing ozone (1 ppm, 4 °C, 120 s) or chlorine (200 ppm) and subsequent washing in tap water (4 °C, 90 s), and storage at 4 °C for up to 9 days	AMB *Pseudomonades* *Enterobacteriaceae* Color Texture Visual appearance Aroma Flavor	Reductions of AMB were similar to those from water washing, but *Enterobacteriaceae* and *Pseudomonades* were strongly reduced No impact on color, texture, visual appearance, aroma, off-flavor and off-odor were achieved	Baur et al. [17]

Table 6 continued

Produce	Treatment conditions	Quality/safety characteristics	Results	References
Lettuce	Immersion in water containing ozone (3 ppm) for up to 5 min, and then storage at 4 °C for 9 days	*E. coli* O157:H7 *L. monocytogenes* AMB Yeasts/molds	Ozone was effective in the inactivation of *E. coli* and *L. monocytogenes* Populations of both pathogens remained relatively unchanged, whereas numbers of AMB and yeasts/molds increased during storage	Rodgers et al. [102]
Lettuce	Combinations of ozone (from 0 to 7.5 ppm) and chlorine (from 0 to 200 ppm)	Natural aerobic flora Shelf life based on appearance	The greatest measured reduction was achieved by combining 7.5 ppm ozone with 150 ppm chlorine, which resulted in a microbial load reduction of 2.5 log CFU/g Lettuce salads treated with chlorine, ozone, or a combination had a shelf life of 16, 20, or 25 days, respectively	Garcia et al. [45]
Lettuce	Washing with water containing ozone at 5.2, 9.7, and 16.5 ppm for 1, 5, 10, and 15 min Combinations of aqueous ozone (9.7 ppm for 10 min), aqueous chlorine dioxide (10 ppm for 10 min) and thyme oil (1.0 ppm for 5 min)	*E. coli* O157:H7	*E. coli* populations were significantly reduced after 10 min washing in treatments with water containing 9.7 or 16.5 mg/L of ozone The lower concentration (5.2 mg/L) did not result in any significant microbial reduction during 1, 5, 10, or 15 min of washing Sequential washing were very effective in killing *E. coli*, especially in the following order: thyme oil/aqueous ClO_2/aqueous ozone	Singh et al. [111]
Lettuce	Soaking in water containing 5 ppm of ozone	AMB Yeasts/molds Coliforms Bacterial spores	Water containing ozone reduced the viable AMB and yeasts/molds by 1.5 and 1.0 log CFU/g, respectively within 10 min Coliforms were reduced to less than 100 CFU/g Bacterial spores were not removed from lettuce surface	Koseki et al. [68]
Lettuce	Bubbling ozone in water for 3 min. Application of stirring (low and high speed), sonication or stomaching, for ozone delivery	Microbial counts	Efficient ozone delivery for microbial inactivation requires a combination of ozone bubbling and high-speed stir	Kim et al. [62]
Onions (green)	Washing in water containing ozone (1) 0.05–0.1 ppm and (2) 0.1–0.2 ppm	AMB *S. enterica* *E. coli*	Ozone washings not significantly reduced AMB, *E. coli* or *Salmonella* counts	Long et al. [71]
Pak Choi (*Brassica rapa*) vegetable	Dipping in water containing ozone (1.4 and 2.0 ppm) at 14 and 24 °C for 15 and 30 min	Pesticides	Low ozone levels shown to be effective in reducing pesticides	Wu et al. [133]
Pak Choi (*Brassica rapa*) vegetable	Dipping in water containing ozone (1.4 and 2.0 ppm) at 14 and 24 °C for 15 and 30 min	Pesticides	The quantity of pesticide being retained by vegetable highly depends on the levels of ozone and temperature	Wu et al. [134]
Peppers (red bell)	Dipping in water containing ozone at 0.3 and 2.0 ppm for 1, 2 and 3 min	*L. innocua*	The highest microbial reductions were obtained for the highest concentration during 3 min (2.8 log-cycles reduction)	Alexandre et al. [9]
Peppers (red bell)	Dipping in water or water containing ozone at 0.3 ppm for 1, 2 and 3 min	*L. innocua*	Ozone generally added an additional reduction of approximately 0.4 log-cycles in comparison with water washings	Alexandre et al. [7]
Pepper (green)	Water containing ozone (4.0 or 8.0 ppm) was sprayed twice everyday (10 a.m. and 2 p.m.) for 3 successive days, under well-ventilated conditions	Visible injury	Results indicated that intensive ozonated water spraying with a high concentration, for airborne-disease control, does not cause any visible injury to the seedlings used, as long as the spraying is carried out under well-ventilated conditions	Fujiwara et al. [43]

Table 6 continued

Produce	Treatment conditions	Quality/safety characteristics	Results	References
Peppers (green)	Washing with water containing ozone (0.30–3.95 ppm) for 20 s, 1, 5, 15 or 30 min	AMB	Washing with water containing ozone (0.72 log reduction) was not found to be significantly more effective than washing with water without ozone (0.66 log reduction)	Ketteringham et al. [60]
Pepper (red)	Dipping in water containing ozone (8.0 ppm) for 30 min	Biothiol content Total bacteria counts	On average, ozone decreased the mean level of biothiols by approximately 44 % 1.65 log reductions were observed	Qiang et al. [96]
Potato (peeled strips)	Washing in (1) water containing ozone (20 ppm) and (2) water containing ozone, followed by dipping in Tsunami solution (300 ppm) up to 5 min, packaging and storage under vacuum or MAP at 4 °C for up to 14 days	Respiration rate Sensorial analysis AMB Total psychrotrophics Coliforms Lactic Acid Bacteria (LAB) Yeasts/molds Total anaerobic bacteria	After vacuum storage, there was no evidence of browning in samples dipped in water containing ozone or ozone plus Tsunami, and these treatments maintained initial texture and aroma. However, the use of water containing ozone alone was not effective in reducing total microbial populations. Ozone plus Tsunami resulted in the most effective treatment to control microbial growth, achieving 3.3, 3.0 and 1.2 log reductions for LAB, coliforms and anaerobic bacteria, respectively	Beltran et al. [20]
Rocket leaves	Washing with water containing ozone (10 ppm), packaging in air and in a modified atmosphere and storage at 4 °C for 15 days	Mesophilic and psychrophilic bacteria Coliforms LAB Yeasts/molds Vitamin C Polyphenols Chlorophylls Glucosinolates Sensorial analysis	The reduction in total mesophilic bacteria, coliforms and yeasts/molds after washing were $\approx$1, 0.5 and 0.5 log, respectively Results showed that optimal MAP conditions after ozone application are extremely important during storage to do not promote visual deterioration followed by significant nutrient loss	Martinez-Sanchez et al. [74]
Spinach	Dipping in water containing ozone (8.0 ppm) for 30 min	Biothiol content Total bacteria counts	On average, ozone decreased the mean level of biothiols by approximately 48 % 0.42 log reductions were observed	Qiang et al. [96]
Watercress	Dipping in water containing ozone at 0.3 and 2.0 ppm for 1, 2 and 3 min	Total coliforms	The highest microbial reductions were obtained for the highest concentration during 3 min (1.7 log-cycles reduction)	Alexandre et al. [9]
Watercress	Dipping in water or water containing ozone at 0.3 ppm for 1, 2 and 3 min	Total coliforms	Ozone generally added an additional reduction of approximately 0.4 log-cycles in comparison with water washings	Alexandre et al. [7]

each combination of commodity/microorganism of concern.

Bacterial spores are known to be extreme resistant to environmental abuses and toxic chemicals. When compared to vegetative cells, bacterial spores showed greater resistance to ozone treatments under several conditions [4, 73]. Although many factors contribute to this resistance, the major contribution appears to be the multilayered spore coat [135].

Food components have also a significant effect on the bacteriocidal power of ozone against bacteria. Guzel-Seydim et al. [48] evaluated the efficacy of ozone (at 0.4 ppm for 10 min) to reduce *Bacillus stearothermophilus* spores, vegetative cells of *E. coli* and *Staphylococcus aureus* in the presence of fat, protein, and carbohydrate sources. They concluded that starch provided little or no protective effects compared to the buffer control, locust bean gum provided an intermediate level of protection, while the caseinate and whipping cream provided the greatest levels of protection to the bacterial populations.

Long et al. [71] verified that produce decontamination can be significantly affected by the type of product, linked to its surface characteristics. The authors noted that the folding and indentations on tomato and green onion surfaces enable bacterial attachment that allows their survival after sanitization treatments.

Fungi Inactivation and Mycotoxins Degradation

Produce decay is mainly associated with fungi such as *Aspergillus* and *Penicillium*. These fungi, together with *Fusarium*, are the main producers of mycotoxins, especially aflatoxins, ochratoxin A, and patulin, frequently found in dried figs, raisins, and fruit juices, respectively.

Ozone has been applied to inactivate these mycotoxigenic fungi and also to degrade their mycotoxins (Tables 4, 5, 6). Freitas-Silva and Venancio [42] gathered information about this subject. They suggested that the mechanism involved in fungi inactivation by ozone is also related with implications in membrane integrity.

As happens to bacteria, the sensitivity of fungi species to ozone differs. Palou et al. [89] observed that, although the growth of *Penicillium italicum* was inhibited by ozone, *Penicillium digitatum* was ozone resistant.

Most of the applications of ozone for fungi inactivation and mycotoxins degradation use ozone in the gaseous state. Zorlugenc et al. [139] compared the effectiveness of gaseous ozone and aqueous ozone on fungi inactivation and on the removal of aflatoxin B1 from dried figs. Results indicated that gaseous ozone was more effective than water containing ozone on aflatoxin B1 reduction, whereas aqueous ozone was found to be more effective in fungi inactivation. These conclusions reinforce that the efficacy of ozone must be assessed for each microorganism of concern.

Cataldo [29] reported the potential of ozone industrial application to eliminate patulin toxin in apple juices.

Pesticide Degradation

Residual pesticides are commonly found in fruits and vegetables, since significant quantities of these substances are often used for crop protection. Some pesticides have been identified as potential chemical mutagens; pesticide residues in fruits and vegetables, such as dichlorodiphenyltrichloroethane, diazinon, and parathion, generate concern due to their potential long-term adverse effects as carcinogenic substances [26]. Therefore, effective techniques for the removal or reduction of pesticides residues on produce should be applied. Ozone has already a long history of investigation in pesticide degradation. More recently, several researchers determined the effectiveness of water containing ozone in the degradation of several pesticides in fruits [83] and vegetables [133, 134]. A significant reduction of pesticides, highly dependent on the dissolved ozone levels and temperature, has been reported. However, care must be taken because ozonation of the pesticides may produce by-products potentially more toxic than the pesticides themselves [54].

Effect of Ozone on Product Quality

Although an ozone treatment can lead to a safer product with an extensive shelf life, the overall quality of the final product, in terms of physico-chemical, sensorial, and nutritional features, must be ensured. Therefore, process conditions must prevent, as far as possible, excessive losses of quality attributes. Several researchers evaluated the effects of ozone (in its gaseous and aqueous form) on the quality of some fruits and vegetables. As occurs in produce decontamination, the ozone effects on quality are also highly influenced by the product (Tables 4, 5, 6). Moreover, if ozone treatments are not properly applied, they can promote oxidative spoilage of the products.

Sensorial Characteristics

Consumers "buy with their eyes" and consequently fresh appearance is the first perceived quality attribute. Appearance, color, aroma, taste, and texture are the key sensorial characteristics that contribute to the overall quality evaluation of a product. These characteristics may be evaluated by a group of testers who have trained their sensory faculties and can describe products on the basis of sight, smell, taste, or touch.

Results of sensorial analysis described in some works [2, 4, 17] did not reveal significant differences between ozonated and non-ozonated tomatoes, dried figs, and lettuce. Wang et al. [129] concluded that cilantro samples treated with ozone exhibited better overall quality retention during storage than samples treated with acidic electrolyzed water. The ozone-washed cilantro leaves preserved their fresh characteristics, with a green and fresh appearance, no yellowing or dehydration, and no trace of off-odor.

Sometimes the results obtained in the sensorial analysis do not correlate with the instrumental ones. As suggested by Rico et al. [101], this might be due to the high variability of the product and/or the limited discriminative ability of human perception.

Color

Color is the primary characteristic of a product that influences the consumer approval. The color alterations are affected by changes in the content of natural pigments, such as chlorophylls, carotenoids, and anthocyanins, and/or by other pigments resulting from enzymatic and non-enzymatic browning reactions.

Generally, no changes in produce color are observed after ozone treatments. However, some exceptions had been reported, especially when high ozone concentrations are applied. Das et al. [34] noted that high concentrations of ozone gas (at 30 ppm) caused an undesirable surface discoloration of tomatoes. In contrast, Forney et al. [40] concluded that low ozone concentrations slightly reduced the rate of yellowing of broccoli, one of the major symptoms of senescence. At higher concentrations, ozone reduced the broccoli yellowing probably by the inhibition of chlorophyll degrading enzymes and/or the induction of antioxidants that can protect chlorophyll. Selma et al. [109] verified deeper color changes in cantaloupe samples stored for long periods treated with higher doses of ozone. Akbas and Olmez [3] reported that for lettuce, and immediately after ozone treatment, the color was not affected. However, and during storage, it was verified a decrease in green color, yellowness, and darkening. According to Bolin and Huxsoll [25], chlorophyll breakdown in the cells may explain the loss of greenness, the decrease in yellowness may be explained by the loss of β-carotene during storage, and lettuce darkening was probably caused by phenolic oxidation and bacterial spoilage over time [3].

Some researchers [65, 70] also detected some discoloration on ozone-treated carrots and spinach.

Color degradation of fruit juices due to ozone processing has also been reported for apple [92, 122], grape [119], orange [116], strawberry [120], and tomato juices [117]. Different compounds are responsible for the color changes, depending on the fruit. For instance, several phenolic compounds, such as flavonols, phloridzin, and hydroxycinnamic acids, can contribute to color characteristics in apple juice [104]. In orange juice, carotenoid pigments contribute to yellow, orange, or red color. In contrast, the changes in strawberry juice are usually associated with anthocyanin degradation, since anthocyanins are responsible for the appealing bright red color [120].

Enzymatic browning causes color alterations by the action of the browning-related enzymes (Polyphenol oxidase—PPO and peroxidase—POD). An inhibitory effect of ozone on these enzymes, probably due to the high oxidation potential of ozone, has been reported [19, 138]. However, such inhibitory effect is commonly observed at the beginning of the storage period [101]. Then, produce browning greatly increases with ozone concentration and storage time. This can be caused by the dissolved O_2 produced from ozone decomposition over time leading to browning of the produce surface.

The color of fruits and vegetables is greatly influenced by the physical state of ozone. Bialka and Demirci [22] studied the color of blueberries subjected to gaseous and aqueous ozone. The color of samples treated with gaseous ozone was significantly different from the color of untreated ones (berries appeared darker). When aqueous ozone was applied, no significant alterations were detected.

Some researchers have been trying to minimize the color losses in products treated with ozone. Whangchai et al. [131] concluded that ozone, in combination with oxalic or citric acids, caused a reduction of PPO activity and consequently less browning occurred on longan fruit. Wang et al. [129] verified that the color of cilantro leaves was not affected by ozone, either after washings treatments or during the entire storage period. The low temperature of storage might have slowed down the degradation of chlorophyll, the major pigment of cilantro leaves.

Aroma

Reduced emission of volatile compounds has been reported as the factor most likely responsible for the loss of aroma in fruits. Several researchers observed reductions in aroma volatiles caused by ozone treatments [40, 41]. Nadas et al. [78] concluded that ozone-enriched cold storage of strawberries resulted in a reversible loss of fruit aroma. The authors suggested that this was caused by the oxidation of the volatile compounds released by the fruit. Perez et al. [94] observed a reduction of 40 % in the emission of volatile esters in ozonated strawberries, which caused a detrimental effect on the aroma. Once alterations in any of the aroma biosynthesis related enzymes (lipoxygenase, hydroperoxide lyase and alcohol acyltransferase) were not detected, the reduction of volatile emission can be explained by a physical alteration of the fruit surface.

Antioxidants

Antioxidants encompass a broad range of substances that interfere with the propagation of free radicals, preventing oxidative food damage. These substances are antibacterial and anticarcinogenic agents and have health-protective action. The antioxidant capacity of fruits and vegetables is mainly due to the presence of vitamin C, anthocyanins, carotenoids, and polyphenols. It is expected that ozone causes the loss of antioxidant compounds due to its strong oxidizing activity. However, the oxidative stress caused by ozone may induce some defense mechanisms in produce [39]. In general, antioxidant defenses include both enzymatic and non-enzymatic systems. Non-enzymatic antioxidants include ascorbic acid (AA), glutathione, *N*-acetyl cysteine, tocopherols (vitamin E), carotenoids, and phenylpropanoids. The major detoxifying enzymes are superoxide dismutases (SOD), catalases (CAT), ascorbate peroxidases (APX), and glutathione peroxidases (GPX) [56]. All these antioxidant substances work as scavengers of reactive oxygen species (ROS), preserving cell structures and biomacromolecules from the oxidative damage.

The direct reaction between ozone and an antioxidant triggers an ozone detoxification process that results from the formation of different ROS, such as hydrogen peroxide (H_2O_2), superoxide radicals ($\cdot O_2^-$), and hydroxyl radicals ($\cdot OH$) inside the plant cell [77].

Non-enzymatic Antioxidants

Vitamin C Vitamin C includes AA and its oxidized form, dehydroascorbic acid. AA is one of the most abundant and important antioxidant in plants that can react with ozone and other active forms of oxygen before they damage plant cells [39]. A variety of interactions between ozone and AA has been reported. Alothman et al. [10] detected a decrease in vitamin C content in pineapple, banana, and guava treated with ozone. They explained this decrease by the scavenging of the free radicals formed during the decomposition of ozone. The activation of ascorbate oxidase may also have contributed to the degradation of AA to dehydroascorbic acid [69].

Some authors observed an increase in AA in fruits and vegetables in response to ozone exposure, explained by an antioxidative system that promotes the biosynthesis of vitamin C from carbohydrate reserves of the plant [94]. However, for fresh-cut celery, Zhang et al. [138] concluded that lower ozone concentrations allowed higher vitamin C retention.

Anthocyanins Anthocyanins degradation is the result of the strong oxidizing potential of ozone. Anthocyanins may undergo oxidative cleavage either by direct interaction with ozone or due to the formation of various intermediate radicals ($\cdot OH$, $HO^2\cdot$, $\cdot O_2^-$, and $\cdot O_3^-$) [120].

Although, in general, the anthocyanin content of a product decreases with the increase in ozone concentration and time of treatment, some anthocyanins are stable at high concentrations of ozone [119]. The anthocyanin content of strawberries [94] and blackberries [16] was only slightly affected by ozone treatments. Under refrigerated storage, Alexandre et al. [6] observed that anthocyanin content was better retained when strawberries were previously treated with ozone.

Polyphenols These compounds include phenolic acids, flavonoids, stilbenes, and lignans, which represent a significant part of the total antioxidants content in fruits and vegetables. The polyphenols content of a product is highly dependent on the product and is affected by ozonation. However, published results on the impact of ozone on polyphenols content are contradictory. Alothman et al. [10] and Rodoni et al. [103] concluded that the presence of ozone induced the accumulation of phenolic compounds, probably by the activation of pre-existing enzymes, such as phenylalanine ammonia-lyase (PAL). This was confirmed by Booker and Miller [27], who found that ozone treatments induced PAL, a key regulatory enzyme in the biosynthesis of phenolic compounds, and resulted in an increased accumulation of caffeic and p-coumaric acids.

In other studies, it was proven that ozonation caused significant degradation of phenolic compounds [31, 92, 122]. The authors alleged that the degradation of polyphenols during ozonation can be the result of several possible chemical reactions. Those reactions may be direct reactions of ozone with the target compound or with its intermediates, and reactions between hydroxyl radicals produced through ozone decomposition catalyzed mainly by the hydroxide ion ($\cdot OH$).

Enzymatic Antioxidants

Enzymatic detoxification of ROS requires the coordinated action of several enzymes, mainly SOD, APX, CAT, and GPX, located both in cell compartments and extracellular space [56]. Higher activities of these antioxidant enzymes may help protecting produce from oxidative stress, slowing down the senescence process.

In fact, An et al. [11] reported higher enzymatic activities in ozone-treated asparagus than in control samples. Boonkorn et al. [28] also observed that SOD activity in the tangerine peel rapidly increased after ozone treatment. They considered that the induction of SOD activity after ozone exposure may inhibit free radical accumulation in tangerine peel. This results in increased dismutation of radicals to hydrogen peroxide, which then changes into non-toxic molecules by the activity of other enzymes such as CAT or APX. In conclusion, they suggested that SOD, CAT, and APX might be mainly involved in protecting the fruit tissues from phytotoxicity caused by the oxidizing effect of ozone, since the high dose of ozone used in the experiments did not cause physical injury on tangerine peel.

Firmness

In the majority of published works, ozone is referred by its efficiency in preserving products firmness [6, 76, 126]. However, and inevitably, product firmness decays over storage time [2, 110] but, in general, ozone treatments have little impact on such degradation. The firmness decrease along storage may be attributed to the tissue injury caused by ozone treatments [129] or reduction of cell turgor due to water loss [3]. Rodoni et al. [103] studied the effect of the ozone treatments on tomato cell wall modifications that could be related to the delay in softening observed. Ozone exposure caused a clear decrease in pectin methylesterase (PME) activity, pectin solubilization, and depolymerization. Alterations of other degrading enzymes of the cell wall or changes in cell wall cross-linkages might have also contributed to cell walls disassembly. Indeed, Hong and Gross [51] postulated that oxidizing agents, such as ozone, could cause oxidation of feruloylated cross-linkages or phenolic cross-linkages among cell wall pectin, structural proteins or other polymers, and thereby change the firmness of the product. Rico et al. [101] also found that PME activity decreased in lettuce upon ozone treatments and correlated that decrease with a lower crispiness observed. Ozone can slow the increase in lignin levels in asparagus [11] and carrots [31]. The toughening observed during storage of these vegetables was probably attributed to the thickening of the structural polysaccharide components of the cell walls.

Wei et al. [130] studied the impact of ozone concentrations, time of exposure, and other factors (temperature and pH) on lettuce firmness. They observed that ozone treatment applied at $pH = 8$ resulted in a substantial decline of lettuce firmness. Thus, they recommended to treat lettuce at a pH not higher than 7.

Total Soluble Solids and Sugar Contents

In the majority of published works, it is not reported significant differences in total soluble solids content between ozonated and untreated samples. However, Alegria et al. [5] noted a significant decrease of total soluble solids content in ozonated carrots, probably explained by a leaching process, once carrots were treated with water containing ozone. Minas et al. [76] also detected a decrease in soluble solids content in kiwifruits, higher than the decrease observed for control samples, stored in an ozone-enriched cold atmosphere.

In general, no significant decreases in total sugar content of fruits and vegetables are observed after treatments with water containing ozone [138] or when short-term gaseous ozone treatment is applied [103]. However, when products are stored in an atmosphere containing ozone, sugar content fluctuations are verified. Perez et al. [94] observed a decrease in the contents of sucrose, glucose, and fructose of strawberries stored in an ozonated atmosphere. The authors explained this occurrence by an activation of other sucrose degradation pathways in response to the oxidative stress that ozone may cause. Forney et al. [41] reported that unlike sucrose, glucose and fructose concentrations increased during storage of ozonated carrots. Aguayo et al. [2] also observed higher fructose and glucose contents in tomato slices kept in an ozonated atmosphere. This fact can be a consequence of the conversion of sucrose into glucose and fructose.

pH and Titratable Acidity

Significant effects of ozone on pH and titratable acidity of fruits and vegetables were not found in literature. The exception was the work of Venta et al. [126], in which it is mentioned a decrease in pH values of tomatoes stored in gaseous ozone.

Weight Loss

Contradictory conclusions have been found in the literature about the effect of ozone on weight loss of fruits and vegetables. Ozone treatments reduced weight loss [76, 78, 103, 126], increased weight loss [40, 88], or have no effect at all [70, 88].

Palou et al. [88] exposed peaches and grapes to gaseous ozone and observed a significant increase in peaches weight loss after storage, but not in grapes. Although no shriveling or other injury symptoms were macroscopically visible, the authors suggested that the fruit cuticle and/or the epidermal tissue were probably damaged by the gas.

Forney et al. [40] also reported an increase in broccoli weight loss and assigned this effect to cuticle degradation. Kechinski et al. [59] studied the effects of water containing ozone on the epidermis of Golden papaya fruit and concluded, based in scanning electron microscope images, that the ozone treatment did not affect the fruit cuticular surface. However, the observed increase in weight loss may be enhanced by ozone membrane damage, which causes increased membrane leakiness.

The lower weight loss verified by Nadas et al. [78] in ozonated strawberries after cold storage disappeared when the fruits were returned to ambient temperature. This effect was probably due to a decrease in strawberries transpiration at lower temperature conditions.

Respiration and Ethylene Production Rates

In the majority of published works, neither respiration nor ethylene production rates were significantly affected by ozone treatment. However, some contradictory findings have been reported. These ambiguous results are probably due to the different conditions applied. Usually, low ozone concentrations have no significant effect on respiration and ethylene production rates during storage. In contrast, at high ozone concentrations, the results are different. Forney et al. [40] observed that although 0.2 ppm of gaseous ozone did not affect respiration and ethylene production, a concentration of 0.7 ppm stimulated respiration and ethylene production in broccoli, causing physiological damage. Zhang et al. [138] also reported no significant differences between respiration rate of fresh-cut celery treated with 0.03 ppm of ozonated water and non-treated samples during storage. However, respiration rate was inhibited by treatment with water containing ozone at 0.08 and 0.18 ppm. They concluded that the efficacy of respiration rate inhibition increased with the increase in ozone concentration in water, showing that aqueous ozone was able to retard tissue metabolism. This is in agreement with the study of Toivonen and Stan [121], in which it is described that ozonation reduces respiration, once samples washed in water containing ozone have lower respiration than the ones that have not been treated. Liew and Prange [70] found a slight increase in CO_2 production in carrots treated with ozone, depending on doses and storage time.

In several studies [2, 41, 76], it was evident that the respiration rate was spurred immediately after the ozone treatment, but only for a short period. This fact may indicate that products undergo physiological injury as a result of the oxidative stress caused by ozone exposure. Nevertheless, the respiration rate decreases significantly in ozone-treated products that are stored in cold conditions for long periods. This may be the result of the inhibition of chilling injury by ozone.

Minas et al. [76] found that ozone also delayed climacteric ethylene rise and totally blocked ethylene production in kiwifruits during intermediate (3 months) and long (5 months) cold storage, respectively. Once ethylene plays an important role in produce senescence, these results revealed that ozone can be a main key on the ripening process delay of some fruits and vegetables.

It has been assessed that the combination of ozone with controlled atmosphere storage is crucial for the inhibition of respiration and ethylene production rates and, consequently, for the control of metabolic and tissue senescence rates. Chauhan et al. [31] showed that a storage atmosphere with low O_2 concentration has restricted respiration and ethylene emission rates, when compared with ozone treatments applied alone. It was evident that products shelf life was greatly extended, keeping the overall product quality.

Final Remarks

All data reported in this review showed that ozone treatments can be a suitable choice for fruits and vegetables preservation. In comparison with the chemical alternatives, ozone has several significant advantages, mainly: (1) is one of the most active and strong oxidative agents; (2) it rapidly decomposes to oxygen, leaving no traces; (3) it does not produce toxic halogenated compounds; (4) its action is very rapid, and (5) it is effective against a wide range of microorganisms. However, the efficacy of ozone on microbial inactivation depends on the type of microorganism, type of product, amount and type of organic matter, temperature, pH, concentration of ozone and its physic state, and contact time.

The optimization of ozone processing conditions must be assessed for a particular fruit and/or vegetable, once quality may be affected. Only a well-balanced impact between safety and quality can lead to favorable outcomes for the majority of products. Therefore, studies on the effect of ozone process conditions in specific products are still required in order to attain safe products (from a microbiological perspective) with high-quality features. Such integrated approaches are essential before launching products into commercial circuits and for the development of reliable products as well.

Although combinations of preservatives and treatments to preserve foods are a common practice, studies that combine ozone with other preservatives to enhance the quality and/or safety of fruits and vegetables are scarce. Therefore, studies that combine additives and preservatives systems with ozone are still challenges, since they can provide alternatives for healthy and safe fruits and vegetables.

Acknowledgments Fátima A. Miller and Teresa R.S. Brandão gratefully acknowledge, respectively, their Post-Doctoral grants SFRH/BPD/65041/2009 and SFRH/BPD/41419/2007 to Fundação para a Ciência e a Tecnologia (FCT) and Fundo Social Europeu (FSE). This work was also supported by National Funds from FCT, through project PEst-OE/EQB/LA0016/2011.

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