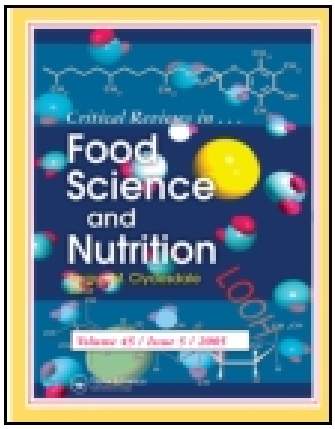

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Application of Ozone for the Postharvest Treatment of Fruits and Vegetables

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Fruits and vegetables consumption has risen noticeably during recent decades, leading to a greater frequency of foodborne illnesses associated with fresh produce. Novel industrial applications and improvements in ozone technology together with new regulatory actions worldwide have emerged in recent years, making its use in the food industry easier. This technology has attracted considerable commercial interest, especially because ozone does not leave any residues on the treated produce and it is also accepted by many organic grower organizations. However, discrepancies regarding the efficacy of this technology are often found in the bibliography and further research is still needed. These differences could be attributed to a great variability in the conditions of the research work: method of ozone generation and application, O_3 concentration and exposure time to the gas, as well as the way in which produce is packed. In this sense, standardization in the working conditions and in the units to measure ozone concentration will be useful to better understand the mode of action and the effects of ozone on food products. Consequently, it would be possible to improve its potential as a sanitizer in the food industry.

Keywords Fresh-cut products, disinfection, alternative sanitizers, extended shelf-life, quality

INTRODUCTION

Fruits and vegetables are important components of the human diet and their consumption has risen noticeably during recent decades (Yuk et al., 2006). This increase in consumption has led to a greater frequency of foodborne illnesses associated with fresh produce (Sivapalasingam et al., 2004) like alfalfa sprouts, lettuce, melon, and prepared salads among others (Han et al., 2002). Therefore, increasing attention has been focused on the safety of these products and, in particular, on the intervention methods to reduce and eliminate human pathogens from fresh fruits and vegetables (Xu, 1999).

Commercially, washing with tap or chlorinated water has been traditionally the most widely used method to reduce the microbial load on both, whole and minimally processed fruits and vegetables (García et al., 2003). However, the effectiveness of chlorination is limited, particularly at high pH or against spore-forming microbes, and its use could result in the potential formation of harmful by-products such as trihalomethanes and haloacetic acids, which can adversely affect human and environmental safety (Xu, 1999; Han et al., 2002; Akbas and Ölmez, 2007). In addition, chlorine may induce off-flavors and alter the taste of the treated fruits and vegetables (Hassenberg et al., 2008).

Regarding synthetic fungicides, many of them are now ineffective because of the proliferation of resistant pathogen strains. Also, there is growing regulatory pressure due to health and environmental concerns related to pesticide toxicity (Palou et al., 2002). As a consequence, there is considerable interest in alternative, safe, but effective, sanitizing agents (Tzortzakis et al., 2007b).

One of these alternatives could be the use of O_3, which has been used as an antimicrobial agent since the late 19th century to purify drinking water (Graham, 1997). At present, it is used in more than 30 different industries due to its high oxidant capacity and because it is effective over a much wider spectrum of microorganisms than chlorine and other disinfectants (Xu, 1999). At the same time, it presents no safety concerns about consumption of chemical residues in the treated food products (Sharma et al., 2003).

Moreover, the use of O_3 yields several advantages over other chemical oxidants; its precursors are abundant and economically advantageous and it can be applied in the gaseous or aqueous state depending on the commodity (Graham, 1997; Guzel-Seydim et al., 2004; Crowe et al., 2007). Besides its antimicrobial activity against a wide range of microorganisms,

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ozone can destroy pesticides and chemical residues and convert nonbiodegradable organic materials into biodegradable forms (Rodgers et al., 2004). In addition, due to its quick decomposition to oxygen and the fact that it does not leave residues on the treated commodities, its application in food processing is allowed by organic certification (Selma et al., 2008b).

In 1997, ozone was granted Generally Recognized as Safe (GRAS) status and; in 2001, it received full US-FDA approval as a direct contact food sanitizing agent (Tzortzakis et al., 2007b).

In the food industry, ozone application has been tested for several purposes like food preservation, extension of shelf-life, equipment sterilization, and elimination of undesirable flavors produced by bacteria during both storage and shipping (Zhang et al., 2005).

Ozone has bactericidal effects on both Gram positive (*L. monocytogenes, S. aureus, B. cereus, E. faecalis*) and Gram negative (*P. aeruginosa, Y. enterocolitica*) bacteria, as well as on spores and vegetative cells. Ozone is also efficient to inactivate yeasts (*C. albicans* and *Z. bacilli*), fungal spores, and a wide range of viruses (Venezuelan equine encephalomyelitis virus, hepatitis A, influenza A, vesicular stomatitis virus, and several bacteriophage strains) (Restaino et al., 1995; Guzel-Seydim et al., 2004).

However, it must be taken into account that exposure to high concentrations of O_3 can cause some detrimental health effects. In the United States, the Federal Occupational Safety and Health Administration (OSHA), and in the United Kingdom, the Health and Safety Executive (HSE, 1996) specify a 0.1 ppm threshold for continuous exposure in the workplace environment during an eight-hour day/40-hour work week period and 0.3 ppm for a 15-minute period (Karaca and Velioglu, 2007). To protect workers from ozone, an effective and reliable ozone monitoring and alarm system must be used and proper personnel protective equipment should be supplied. What is more, if it is improperly used and, due to its strong oxidizing activity, O_3 may also cause some deleterious effects on the treated produce such as physiological injury and oxidative stress in plant tissues (Forney, 2003; Karaca, 2010). Some of the symptoms of these injuries are browning, pitting, increased decay and weight loss and, fruit discoloration among others (Forney, 2003).

The aim of this article is an in-depth and updated review of the studies based on ozone applications on fruits and vegetables, both as whole and minimally processed products. Other products such as mushrooms, black peppers, and alfalfa sprouts are also presented.

OZONE APPLICATIONS IN FRUITS AND VEGETABLES

For the postharvest treatment of fresh fruit and vegetables, ozone can be either used as a prestorage or storage treatment in air or water, or it can be added as a continuous or intermittent component of the atmosphere throughout storage or transportation (Palou et al., 2002).

The efficiency of ozone in microbial inactivation depends on a number of factors: the type and physiology of the treated product, the reactor design, the water quality, temperature and pH, and the ozone demand of the medium (Kim et al., 1999; Karaca and Velioglu, 2007). In this sense, impurities in the water or the organic matter present in the treated medium react with ozone, decreasing its sanitizing power (Block, 1982). Thus, the effectiveness of this gas will depend not only on the amount applied, but more on the residual ozone, which is the detectable concentration, in the treatment medium, after it has been applied to the targeted food product (Karaca and Velioglu, 2007).

Another parameter that should be considered is the initial microbiological load and kind of contaminating microorganisms on the products: the higher the microbial load, the lower the effectiveness of O_3 may be. This is due to the fact that the gas eliminates competitive microflora, but at the same time, ozone is continuously degrading to O_2 and, therefore, it may enhance the aerobic microorganisms' counts.

For both, gaseous and aqueous applications, safety aspects must be taken into consideration. If gaseous O_3 is used in cold stores or closed places, concentrations must be precisely monitored and appropriate safety intervals before opening the treated rooms must be established in order to avoid personnel health risks.

When O_3 is used in aqueous phase, the excess undissolved gas must be destroyed or converted back into oxygen before being released into the atmosphere. Small heated catalyst ozone scrubbers are usually installed for this purpose (Pascual and others, 2007).

Gaseous Ozone

Gaseous ozone is a strong sanitizer and can be used to disinfect storage rooms, to prevent bacteria, molds, and yeasts development on the food surfaces, to control insects, and to degrade mycotoxins. It can also eliminate undesirable flavors produced by bacteria and chemically remove ethylene gas to slow down the ripening process (Rice and others, 1982).

When ozone is used at proper concentrations in the storage atmosphere, several fruits and vegetables can be protected by the gas against diseases and with minimum physiological damage (Nadas et al., 2003). In low concentrations it is not extremely toxic, but at high concentrations it may be fatal to humans affecting primarily the respiratory tract (Guzel-Seydim and others, 2004).

Ozone gas efficacy to inactivate microorganisms is conditioned by the species considered, its growth stage, the ions present in the air, the O_3 concentration and exposure time, and the temperature and relative humidity of the room (Pascual and others, 2007). In air, the reactivity of ozone is greatest with fungi, molds and some odor-causing chemicals and least with dry spores and bacteria.

For optimum efficiency, it is also essential that the gas is thoroughly and evenly distributed quickly. Otherwise, decomposition will occur before the O_3 is able to contact its target (Rice et al., 1982).

When it is used on fresh produce, in general, fruits are more tolerant to O_3 than vegetables and, therefore, higher concentrations can be used on the former without risk of damage (Forney, 2003). On the other hand, for surfaces and equipment disinfection, O_3 is less effective in air than in water and, consequently, higher concentrations or longer application times are needed when O_3 is applied as a gas than when ozonated water is used: 1–4 hours versus 1–10 minutes, respectively (Rice and others, 1982). Conversely, ozone has a longer half-life in the gaseous state than in aqueous solution: about 12 hours in air and only 20 minutes in water at 20°C (Graham, 1997) with faster decomposition at higher water temperatures (Rice and others, 1982).

Aqueous Ozone

Ozonated water can be used for sanitation of the surfaces of packaging equipment, sanitation of water in washing systems, dump tanks or flumes, and during the postharvest washing of fruits and vegetables to control infections by decay pathogens or their propagules (Smilanick et al., 2002; Mahapatra et al., 2005). In this case, aqueous O_3 is used as an alternative to chlorine to sanitize the water used to cool or wash fruits and vegetables, improving the microbiological quality by controlling the pathogens on the surface of these products (Parish and others, 2003) and preventing the build-up of microorganisms (Forney, 2003). O_3 decomposes quickly to oxygen, leaving no residues and it has more potency against bacteria, cysts of protozoa, viruses and fungal spores than hypochlorite (White, 1992). However, as ozone washes are primarily surface treatments, they are not effective in reducing decay of wound-inoculated fruit, where pathogens appear to be well protected from oxidation by ozone, probably as a result of limited penetration due to the reaction of ozone with reactive compounds present in the wound. What is more, the presence of dirt, soil, fruit residues or any organic material in the wash water can protect the microorganisms from being inactivated by ozone (Karaca, 2010).

Ozone is only partially soluble in water and its solubility increases as the temperature decreases (Xu, 1999). Even when its maximum solubility at 20°C is 29.9 μg/mL, in practice it is difficult to exceed 10 μg/mL (Smilanick and others, 1999) and ozone in water above 1 μg/mL can liberate ozone into the air that exceeds safe recommended levels. Furthermore, achieving high dissolved ozone concentrations in water is costly and difficult to stabilize (Ölmez and Kretzschmar, 2009). These researchers stated that in the processing industry, it is also important to keep the applied ozone levels as low as possible to avoid the corrosion of stainless steel which increases when O_3 concentration is above 1 ppm.

The rate of ozone solubilization also increases when purity of water and pH values increase and decrease, respectively. High pH and the presence of minerals in water may destabilize ozone, enhancing its decomposition (Khadre et al., 2001). Kim et al. (1999) reported the greatest stability of ozone in solution with a pH of 5, while no ozone was detected when the pH increased up to 9. In pure water, it rather quickly degrades to oxygen, and even more rapidly in impure solutions (Hill and Rice, 1982) where impurities react with and consume the applied ozone.

These difficulties in the solubilization of ozone may be, at least partially, responsible for the lack of consistency in the concentrations obtained in different studies and, thus, in the disagreement found in the bibliography on the effectiveness of ozonated water (Beltrán et al., 2005a). According to these authors, in terms of process optimization and knowledge of shelf-life application, an ozonating system should include an ozone analyzer, an ambient sensor, a probe, a temperature controller, an effective system for dissolving ozone gas in the water, and a recirculation system that supplements new ozonated water.

In addition to reducing microbial contamination, after the treatment with O_3, the wash water can be recycled, reducing water usage as it is free of chemical residues. Ozone can destroy pesticides and chlorinated by-products and effectively oxidize chemical contaminants in water, including those responsible for odors and discoloration as well as iron, manganese, and sulfur without producing any by-products of human health concern (Forney, 2003).

In the following sections, the applications of O_3 on whole and minimally processed fruits and vegetables and, in both gaseous and aqueous phases, are presented.

FRUITS

The sensitivity of a fruit to ozone has been found to vary according to fruit types and even according to species within a given fruit type (Rice et al., 1982). This should be taken into account before a treatment with ozone is decided and, in order to avoid damage to the produce, the best conditions for the application must be determined for each particular commodity.

Berries

Early studies indicated that the shelf-life of different berries like strawberries, raspberries, currants, and grapes could be doubled by applying 2–3 ppm of gaseous ozone continuously or for "several" hours per day (Ewell, 1950), but no specification is given on the duration of the intermittent treatments. On the other hand, other authors worked with diverse berries and did not find positive effects of an O_3 application (Spalding, 1966; Norton et al., 1968; Spalding, 1968). These discrepancies could be attributed to differences in the gas concentrations used, the temperature at which the O_3 was applied, the delivery system, and lack of accuracy in determining O_3 concentration in the storage rooms. In the following sections, the results of several researches on berries will be summarized.

Blackberries

Barth et al. (1995) reported that a continuous supply of 0.3 ppm gaseous O_3 resulted in an extension of the market quality of "Chester" blackberries in comparison with fruits stored

in air. The treatment with O_3 was very effective in preventing fungal decay, caused mainly by *B. cinerea*, and in maintaining the red color of the blackberries for up to 12 days of storage at 2°C. Moreover, those authors found that the anthocyanins' content and the fruit quality were not affected by exposure to the gas while the peroxidase (POD) enzyme activity, which is implicated in discoloration of plant products, remained lower in the O_3-stored fruit than in the control ones.

Blueberries

Several studies were conducted with blueberries with somehow contradictory results which could, at least partially, be explained by differences in the storage conditions after the O_3 treatment. In this sense, and as it is described below, maintaining low temperatures as well as the use of controlled atmospheres (CA) enhanced O_3 positive effects on blueberries' quality, while the opposite occurred when the fruit was kept at higher temperatures.

On the one hand, Spalding (1968) observed that an application of 0.5 ppm O_3 for two days at low temperature followed by four days at 21°C or six days at 15°C did not influence the subsequent shelf-life of blueberries with similar percentages of rotted fruits as the control group. On the other hand, Song et al. (2003a) stated that blueberries treated with 700 ppb of O_3 for four days followed by storage for four weeks in CA maintained a significant higher marketability than the control group, indicating that O_3 application could be used to reduce decay of blueberry fruit.

Likewise, Crowe et al. (2007) found that spraying blueberries with water containing 1 ppm O_3 was also effective in reducing microbial counts on fruit inoculated with *P. fluorescens* and *E. agglomerans*. *P. fluorescens* population decreased by 2.57 and 2.80 log units for treatments of 60 and 120 s, respectively. For *E. agglomerans*, the reductions observed after O_3 treatment were >2.3 log and no enhancement in the antimicrobial effectiveness was obtained by increasing the length of time reaction or by combining the O_3 with hydrogen peroxide. For both pathogens, the reductions obtained with the O_3 treatment were significantly higher than those achieved with chlorine and water (approx. 1 log).

Regarding physicochemical parameters, ethylene production, respiration quotient, and the most abundant esters produced by the blueberries were not affected by exposure to 700 ppb of O_3 for four days (Song et al., 2003a). These authors did not observe phytotoxicity symptoms, but the production of limonene was reduced and, at the same time, the synthesis of methanol, ethanol, and 2-nonanone was induced by O_3 treatment, implying that O_3 may have caused some stress in the fruits. However, antioxidant capacity, anthocyanins, and phenolic compounds were not significantly increased by treatment with O_3.

Cranberries

Norton et al. (1968) investigated the effects of the application of gaseous O_3 on the quality of "Early Black" and "Howes" cranberries. These authors concluded that the treatment with 0.27 ppm O_3 enhanced weight loss and was not effective in reducing the percentage of rotted cranberries during eight weeks of storage at 4.4°C. By increasing O_3 concentration and storage temperature to 0.60 ppm and 15°C, respectively, damage to the fruit and weight loss were also increased. In these conditions, O_3-treated fruit presented 25% less lipids than the control group in their cuticles and degradation of the cuticle was observed, which in turn could enhance fungal rot and weight loss of the berries. However, it should be taken into consideration that the temperature used was much higher than the recommended for small fruit storage, causing a synergistic effect with the O_3 in the cranberries deterioration.

Raspberries

Bialka and Demirci (2007a, 2007b) reported that the treatment with both, gaseous and aqueous ozone (5% wt/wt in a flow air rate of 0.34 m^3/h) applied for 64 minutes, was effective in reducing the populations of *Salmonella* and *E. coli* O157:H7 on inoculated raspberries without affecting the color of the fruit. Those researchers stated that for *Salmonella* it was possible to achieve reductions in the microbial counts of 1.6 and 4.5 log CFU/g for gaseous and aqueous ozone, respectively. In the case of *E. coli* O157:H7, the counts were reduced by 2.6 and 5.6 log CFU/g for O_3 applied in air and water, respectively. On the other hand, when the fruit was washed with tap water, the maximum decreases in the populations of the studied pathogens were of 1.3 and 1.1 log CFU/g for *E. coli* O157:H7 and *Salmonella,* respectively. When O_3 was applied in the gaseous phase and for both pathogens, the combination of 64 minutes of continuous exposure to O_3 + 64 minutes of exposure to pressurized ozone resulted in a greater efficacy, achieving with these conditions reductions of 3.56 and 3.8 log units for *Salmonella* and *E. coli* O157:H7, respectively. However, the duration of this treatment seems to be excessive to be of commercial interest. In the same way, a 64-minute immersion in water could be detrimental for the quality of this delicate fruit and it would be important to investigate other quality parameters such as firmness and juice leakage which could be affected by this treatment.

Strawberries

The effects of O_3 on spoilage and pathogen microorganisms on strawberries were studied in several researches with nonuniform results. In early studies, Spalding (1968) observed that storage for seven days in an atmosphere with 0.5 ppm O_3 was not effective in reducing the percentage of *Botrytis* or *Rhizopus* rot. Similarly, Allende et al. (2007) described similar results for O_3-treated and untreated strawberries. In effect, these authors did not find significant differences in the final counts of mesophilic and psychrophilic bacteria and molds and yeasts of the control samples and those treated with 5000 mg O_3/L gas carrier stored in either air or active modified atmosphere packaging (MAP) for up to 12 d. In addition, Pérez et al. (1999)

reported that strawberries treated with 0.35 ppm O_3 for three days at 2°C presented, after two days at 20°C, 15% less fungal decay than the control, but after four days at room temperature rot incidence was similar in all the samples.

On the contrary, cold storage of naturally infected "Camarosa" strawberries for three days in air enriched with 1.5 μL O_3/L reduced decay incidence of *Botrytis cinerea* on these fruits when compared with air storage (Nadas et al., 2003). Furthermore, O_3 was also demonstrated to be effective against some pathogens. According to Rodgers et al. (2004), *E. coli* O157:H7 and *L. monocytogenes* were no longer detected and remained undetectable during nine days of storage at 4°C after washing inoculated strawberries for 5 minutes with water containing 3 ppm of O_3. Those authors also reported decreases of approx. 4 to 5, 1 and 2.5 log units for mesophilic bacteria, yeasts, and molds, respectively, immediately after the exposure to O_3. However, all the groups increased during storage with final counts for molds and yeasts significantly higher than the initial populations.

In another set of experiments, strawberries inoculated with *Salmonella* and *E. coli* O157:H7 were treated for 64 minutes with either gaseous (Bialka and Demirci, 2007a) or aqueous (Bialka and Demirci, 2007b) O_3 at a concentration of 5% wt/wt in a flow-air rate of 0.34 m^3/h. Both treatments reduced the pathogens populations with maximum decreases of 0.9 and 1.8 log CFU/g, for *Salmonella* and *E. coli* O157:H7, respectively, for O_3 applied in air and, 3.3 and 2.9 log CFU/g, for washing with ozonated water at 20°C. The combination of continuous gaseous and pressurized O_3 increased the reductions to 2.6 and 2.9 log CFU/g, for *Salmonella* and *E. coli*, respectively.

The effects of O_3 were also evaluated on the physicochemical, sensory, and nutritional quality of strawberries with reported positive and negative results. In early studies, Spalding (1968) noted that the shelf-life of strawberries was not enhanced by storage in an atmosphere with 0.5 ppm O_3 compared with fruits stored in air. Moreover, the strawberries were injured by the O_3, causing the caps to shrivel and dry. Published results concerning vitamin C content are also contradictory as fruits treated with O_3 presented higher, lower or similar concentrations of vitamin C than the controls. On the one hand, Pérez et al. (1999) reported that fruits stored three days in an atmosphere with 0.35 ppm O_3 presented lower levels of glucose and fructose than the control and, at the same time, vitamin C content was three-fold higher than that of control fruit. The authors attributed these changes to the activation of an antioxidative system that promoted the biosynthesis of vitamin C from carbohydrate reserves of the fruits as a response to the high oxidative capacity of O_3. On the other hand, Kute et al. (1995) did not find significant differences in total ascorbic acid activity due to treatments with 0.3 and 0.7 ppm O_3. Furthermore, the O_3-treated fruits presented the highest soluble solid contents after seven days of cold storage. Finally, Allende et al. (2007) observed that fruits exposed to 5000 mg O_3/L gas carrier showed the lowest vitamin C contents at the end of a 12-day storage period.

Fruit surface color was not affected by treatments with either gaseous or aqueous O_3 (Nadas et al., 2003; Bialka and Demirci, 2007a, 2007b). What is more, Rocculi et al. (2005) stated that sepals of samples washed for five minutes with 1.66 ppm ozonated water maintained their initial green color better than the control group washed with tap water. Related to firmness, those researchers did not observe significant differences in this parameter between the O_3-treated and the untreated strawberries after 20 days of cold storage. On the contrary, Nadas et al. (2003) demonstrated that the exposure to 1.5 μL/L O_3 for three days at 2°C reduced weight loss and fruit softening with respect to the air-stored fruit.

Another parameter studied was the total phenolic and ellagitanin contents of the fruits. These compounds were similar in O_3-treated and untreated samples after a storage period of 12 days. After cold storage, O_3 affected mainly the procyanidins and anthocyanins contents, which were reduced by the exposure to the gas (Pérez et al., 1999; Allende et al., 2007).

Related to aroma, this is an important quality parameter of strawberries. Nadas et al. (2003) associated the loss of fruit aroma detected immediately after the cold storage period with the enrichment of the atmosphere with O_3. Similar results were reported by Pérez et al. (1999), who observed that treatment with 0.35 ppm ozone produced a reduction of 40% in the emission of volatile esters in strawberries during storage at 2°C. However, these authors did not find clear differences in any of the aroma biosynthesis related enzymes (lipoxygenase (LOX), hydroperoxide lyase (HPL), and alcohol acyltransferase (AAT)) activities between the control and the O_3-treated fruits, and even an increase in the aroma of strawberries was noted upon ozone treatment (Ewell, 1950). Nevertheless, neither reference to the O_3 concentration used nor the duration of the treatment was mentioned in this report.

Finally, in the sensory analysis no significant differences in sweetness, redness, firmness, or juiciness were found between O_3-treated and control strawberries. At the same time, off-flavors were not detected in any of the analyzed fruits (Nadas et al., 2003; Rodgers et al., 2004; Allende et al., 2007).

Table Grapes

Probably, most of the researches on O_3 effects on fruits were conducted on table grapes. In general, the application of O_3 during the storage of table grapes was effective against native superficial microflora, but ineffective when the microorganisms were inoculated or already established on the fruits. In this sense, differences in the effectiveness of the sanitizing treatment might be influenced by the absence of competitive microflora and a higher microbial load of the target pathogens when the fruits are artificially inoculated.

In early studies, the treatment with 0.5 ppm ozone was found to inhibit superficial mold growth after seven days of storage at 15.6°C, but it was not effective to control rot caused by *B. cinerea* on inoculated "Thompson Seedless" and "Tokay" table grapes (Spalding, 1968). Similarly, Palou et al. (2002) demonstrated that gray mold incidence was not reduced by exposure to 0.3 ppm O_3 during seven weeks of cold storage

on inoculated "Thompson Seedless" table grapes. Conversely, nesting and sporulation of the fungus were prevented and, according to these authors, this is important to reduce the amount of inoculum available for reinfections of the stored produce and could be useful to prevent the proliferation of fungicide-resistant strains of the pathogens.

On the contrary, in a set of experiments carried out by Sarig et al. (1996), a supply of 8 mg O_3/min was reported to effectively eliminate the native populations of molds, yeasts, and bacteria in "Alphonse Lavallée" and "Thompson Seedless" grapes after 20 and 40 minutes' exposure, respectively. At the same time, the microflora on cv. "Zeiny" was less affected by the treatment. O_3 application for 30 to 40 minutes was also sufficient to eliminate decay caused by *Rhizopus stolonifer*, in inoculated grapes. Furthermore, O_3 elicited the production of resveratrol and pterostilbene, which, together with the direct fungicidal effect of the gas, contributed to enhance O_3 effectiveness to control decay (Sarig et al., 1996). These authors concluded that a short-term postharvest exposure of grapes to O_3 might be a commercially satisfactory alternative to SO_2 during cold storage, as it was effective in controlling decay and maintain the quality and freshness of the treated fruit superior to those of the control ones for up to nine weeks at 0°C.

Also, Cayuela et al. (2009) observed that addition of 2 ppm O_3 continuously or intermittently (12 hours per day) to the storage atmosphere, was effective in significantly decreasing the decay percentage of three table grapes cultivars ("Superior Seedless," "Regina Victoria," and "Cardinal CL80") after 72 days of cold storage at 5°C compared to fruits stored in air.

Finally, Mlikota-Gabler et al. (2010) also studied the combination of short-term fumigations (60 minutes) with high concentrations of O_3 during pre-cooling of table grapes. These authors suggested that 2500 or 5000 μL/L × h O_3 were equally effective in reducing gray mold by approximately 50% after 7 days of storage at 15°C ("Thompson Seedless") or after 28 days at 0.5°C ("Redglobe") following ozone fumigation. Fumigation with 5000 μL/L O_3 for 60 minutes in a commercial O_3 chamber reduced the incidence of gray mold by approximately 50% among "Autumn Seedless" and "Black Seedless" and by 65% among "Redglobe" grapes during six weeks of storage at 0.5°C (Mlikota-Gabler et al., 2010). The authors attributed the variability in ozone effectiveness to differences in cultivars' natural resistance to gray mold.

In these experiments, decay caused by *Alternaria* and *Penicillium spp*. and infections caused by *B. cinerea* already established in the tissues were poorly controlled by the O_3 treatment (Mlikota-Gabler et al., 2010). According to these researchers, the high ozone demand of the grapes could explain the poor results to control postharvest gray mold observed in previous works with constant low doses of ozone (Palou et al., 2002; Artés-Hernández et al., 2003, 2007).

Researches with O_3 also included studies on the effects of the gas on physicochemical and sensorial parameters and antioxidant compounds of table grapes. Palou et al. (2002) published that after four weeks of storage at 5°C and one week at 20°C, "Flame Seedless" grapes treated with 0.3 ppm O_3 did not present visible injuries in their tissues and weight loss was similar in both, the control and the ozonated rooms. Similarly, Cayuela et al. (2009) described similar weight losses for the control samples and the grapes of three cultivars treated with either continuous or intermittent 2 ppm of O_3 during 30 days of storage at 5°C. However, from this date till the end of the 72-day storage period, both O_3 treatments induced a significant increase in weight loss with respect to the control group stored in air.

Ozone supplied at a rate of 8 mg/min in an air flow of 500 mL/min did not produce phytotoxicity on berries, stems or pedicels of either green or red cultivars in exposures lasting up to 40 minutes. However, longer expositions caused problems on some cultivars. O_3 treatments did not adversely affect the firmness of some cultivars even after 80 minutes of exposure (Sarig et al., 1996) or even had a positive effect in others, in which intermittently O_3-treated fruits showed the lowest firmness losses during storage (Cayuela et al., 2009).

Ozone fumigation did not cause injury to the berries, but the rachis of "Thompson Seedless" grapes were sometimes harmed with development of thin longitudinal darkened lesions and the same was observed on the rachis of small grape clusters immersed for up to six minutes in water with 10 μg/mL O_3 (Mlikota-Gabler and Smilanick, 2001). However, this injury appeared irregularly and was not always associated with a particular ozone dose or grape cultivar (Mlikota-Gabler et al., 2010).

Artés-Hernández et al. (2003) reported that neither intermittent nor continuous exposures to O_3 (8 ppm shocks of O_3 applied 30 minutes every 2.5 hours and 0.1 ppm of the gas, respectively) affected the physicochemical quality of cv. "Napoleon" table grapes during 38 days of storage at 0°C + 6 d of shelf-life at 15°C in air. Only slight still nonsignificant changes with respect to harvest values were recorded for color, firmness, total SSC, pH, and acidity. However, severe stem browning was detected in the grapes subjected to the shocks of O_3 treatment, and this was attributed to the low RH reached in the cold room. Similar results were observed on the physicochemical attributes of "Autumn Seedless" table grapes stored for 60 days at 0°C and 90% RH and exposed to O_3 in continuous or intermittent applications. In both cultivars, weight loss, and decay caused by *B. cinerea* were similar in the O_3-treated grapes and the control samples (Artés-Hernández et al., 2003; 2004, 2007).

On the contrary, total sugars and organic acid contents of "Autumn Seedless" table grapes were not affected by continuous exposure to 0.1 μL/L of O_3 during two months at 0°C and 90% RH. Furthermore, these berries received higher punctuations in the sensory evaluation (browning of the rachis, softness, and visual appearance) than the control samples (Artés-Hernández et al., 2004). On the other hand, off flavors were not detected in any of the cultivars tested during 72 days of cold storage in air or air plus 2 ppm O_3, but the control samples presented significantly better flavor scores than both O_3 stored fruit (Cayuela et al., 2009).

After the shelf-life period, "Napoleon" grapes subjected to intermittent shocks of O_3 during cold storage maintained the total anthocyanin and flavonol concentrations and exhibited an

increase in total stilbenoids, mainly resveratrol and piceid contents. At the same time, in most of the other treatments tested (MAP, CA, and air with or without SO_2, hexenal and hexanal) anthocyanins and flavonols declined and stilbenoids remained with no changes (Artés-Hernández et al., 2003).

Concerning the cv. "Autumn Seedless," O_3 applied either continuously or on an intermittent basis increased the total polyphenol content by 22.8% in comparison with the values found at harvest without causing browning of the tissues (Artés-Hernández et al., 2007).

After 72 days of storage, Cayuela et al. (2009) found the lowest and highest resveratrol content in grapes stored under permanent and intermittent presence of 2 ppm O_3, respectively. Those authors attributed these differences to the fact that the continuous exposition to O_3 could reduce the levels of resveratrol produced as a defensive response of the grapes against the oxidative stress, while the intermittent treatment might be enough to induce the biosynthesis of resveratrol, but would not cause its depletion.

Similarly, the treatment of "Superior" white table grapes with either 3.88 or 1.67 g/h O_3 for one, three, and five hours also induced an increase in stilbenoid and viniferins biosynthesis during storage at 22°C and 95% relative humidity, with the maximum resveratrol concentration reached after two days of storage. Nevertheless, this treatment affected negatively the sensory quality of the grapes due to browning development in the skin after 48 hours of storage (González-Barrio et al., 2006).

The studies on ozone effects on microbial, physicochemical, and sensorial quality of berry fruits are summarized in Table 1.

Pome Fruits

In general, most of the research works on O_3 application on pome fruits are mainly focused on the microbiological quality of apples and pears and on superficial scald control, while the inclusion of studies on physicochemical, sensorial, and nutritional parameters would contribute to a better understanding of the global effects of O_3 on these fruits.

Apples

Similarly to berries, both positive and negative results were reported for the effects of O_3 during storage of apples. Bazarova (1982) reported a reduction in both weight loss and spoilage of the fruits after the exposure of the apples to 5–6 mg/m^3 ozone gas for four hours each day. Likewise, by applying 2–3 cm^3 ozone/m^3 of air some "few hours" a day, it was possible to achieve an extension in the shelf-life of apples of several weeks, but no reference about any specific duration of the treatment is given in this research work. On the other hand, when the O_3 concentration was increased to 10 cm^3/m^3, it resulted in apple damage (Kuprianoff, 1953).

The application of 1–3 ppm O_3 lowered the spore counts and controlled surface molds on packages and walls of apple storage rooms (Smock and Watson, 1941; Schomer and McColloch, 1948). These authors found that ozone prevented aerial growth of the fungus and considerable spreading of spores and retarded the enlargement of infected areas in the fruit itself. However, it was ineffective in retarding the decay of the apples once it began, and in preventing rotting of inoculated or injured fruit. Furthermore, with 3.25 ppm O_3, the gas caused injury to the fruit, altered its flavor and the cuticle of some varieties became sticky and varnish-like. The time of appearance and the extent of the injury varied with the variety. On the other hand, none of these detrimental effects was found when the gas concentration was reduced to 1.95 ppm (Schomer and McColloch, 1948).

Thus, washing inoculated whole and sliced "Golden Delicious" apples with tap water did not affect yeast counts. At the same time, *E. coli* O157:H7, *L. monocytogenes,* mesophilic bacteria, and mold populations were decreased by this treatment by approximately 1 log (Rodgers et al., 2004). In contrast, washing for five minutes in water containing 3 ppm of O_3, reduced both pathogens to undetectable levels after the treatment, while decreases of approximately 4 to 5, 1 and 2.5 log units were found for mesophilic bacteria, yeasts and molds, respectively. In this study, *E. coli* O157:H7 and *L. monocytogenes* remained below the detection limit during the nine days of storage at 4°C. On the contrary, mesophilic bacteria, mold, and yeast populations increased during storage to slightly (yeasts) or significantly (molds) higher levels than the initial contamination, except for whole apples in which mold populations increased only 2 log during the refrigerated storage period.

Achen and Yousef (2001) evaluated the efficacy of O_3 to reduce *E. coli* O157:H7 on inoculated apples by washing the fruit in water with bubbling O_3 or by dipping the apples in ozonated water for one, three, and five minutes. Mean residual O_3 concentration was 25 mg/L. Maximum decreases in the pathogen population on the fruit surface were 3.7 and 2.6 log CFU/g for the washing and dipping treatments, respectively, compared to unwashed control samples. Counts in the stem-calyx region only decreased 0.6 and 0.5 log CFU/g for the bubbling ozone wash and dip method, respectively. This was attributed to the attachment of the inoculated bacteria to the rough surfaces of the stem-calyx area or the inaccessibility of the microorganism in this region to the action of the sanitizer. Differences in counts among the three exposure times (one, three, and five minutes) in both delivery methods and the three tested temperatures (4, 22, and 45°C) were not significant.

Another effect of treatments with 2–4 ppm ozone used for several hours each day during a five-month storage period included the elimination of off-odors caused by surface molds and bacteria (Smock and Watson, 1941). These authors also found that after five months of storage at 4.4°C with 1–2 ppm O_3, ripening of "Rhode Island Greening" apples was delayed together with better firmness maintenance and less scald incidence of the O_3-treated apples with respect to the control samples (Smock and Watson, 1941).

Finally, sensory quality of whole and sliced apples was not affected by washing with 3 ppm ozonated water, with no

Table 1 Ozone treatment studies on berry fruits

Fruit	O_3 Treatment	Parameters studied	Reference
Blackberries	0.3 ppm **gaseous** O_3	Color retention, anthocyanins Fungal decay	Barth et al., 1995
Blueberries	0.5 ppm O_3 **gas**—2 d at low T	Rotting	Spalding, 1968
	700 ppb **gaseous** O_3 + CA	Anthocyanins and phenolic compounds Antioxidant capacity Decay incidence Esters and volatiles Ethylene production Respiration quotient	Song et al., 2003a
	Spraying **water** with 1 ppm O_3	*P. fluorescens* and *E. agglomerans* inhibition	Crowe et al., 2007
Cranberries	0.27 ppm **gaseous** O_3—4.4°C	Weight loss	Norton et al., 1968
	0.60 ppm **gaseous** O_3—15°C	Rotting	
Raspberries	**Aqueous** and **gaseous** O_3	Color	Bialka and Demirci, 2007a, 2007b
	Flow air rate of 0.34 m^3/h with 5% O_3—64 min	Inoculated *Salmonella* and *E.coli* O157:H7 inhibition	
Strawberries	0.5 ppm **gaseous** O_3—7 days	*Botrytis* and *Rhizopus* rot Effects on shelf-life	Spalding, 1968
	5000 mg **gaseous** O_3/L—12 d	Microbial growth Phenolic compounds Ellagitanin contents Sensory analysis Vitamin C content	Allende et al., 2007
	0.35 ppm O_3 **gas**—3 d at 2°C	Aroma – Volatiles Fungal decay Phenolic compounds Vitamin C and sugar content	Pérez et al., 1999
	1.5 μL/L **gaseous** O_3—3 d	Aroma *Botrytis cinerea* incidence Color, firmness, weight loss Sensory analysis	Nadas et al., 2003
	0.3 and 0.7 ppm O_3 **gas**—7 days	Vitamin C, SSC	Kute et al., 1995
	Aqueous and **gaseous** O_3	Fruit surface color	Bialka and Demirci, 2007a, 2007b
	Flow air rate of 0.34 m^3/h with 5% O_3—64 min	Inoculated *Salmonella* and *E.coli* O157:H7 inhibition	Rodgers et al., 2004
	Aqueous O_3: 3 ppm O_3—5 min	Microbial inhibition Sensory analysis	
	Aqueous O_3: 1.66 ppm—5 min	Color, firmness, TA	Rocculi et al., 2005
Table grapes	**GASEOUS** O_3		
	0.5 ppm O_3—7 days	*Botrytis cinerea* incidence	Spalding, 1968
	0.3 ppm O_3—7 weeks of cold storage	Grey mold incidence Weight loss, physiological damage	Palou et al., 2002
	8 mg O_3/min in an air flow of 500 mL/min—20 to 40 min	Microbial growth Phenolic compounds Phytotoxicity symptoms	Sarig et al., 1996
	2 ppm—continuous or intermittent (12 h/d)	Decay Sensory analysis Resveratrol content Weight loss	Cayuela et al., 2009
	2500 and 5000 μL/Lxh—60 min	Grey mold, *Alternaria* and *Penicillium* spp incidence	Mlikota-Gabler et al., 2010
	0.1 ppm O_3—continuous 8 ppm—30 min every 2.5 h	Anthocyanin and flavonol contents *Botrytis cinerea* incidence Physicochemical quality Weight loss Sensory evaluation Sugars and organic acids	Artés-Hernández et al., 2003, 2004, 2007
	1.67 and 3.88 g/h O_3—1, 3, 5 h	Phenolic compounds Sensory quality	González-Barrio et al., 2006

differences observed by the panelists with respect to the control group (Rodgers et al., 2004).

Pears

Spotts and Cervantes (1992) evaluated the efficacy of a line spray of ozonated water (3.1 μg O_3/mL, about five seconds) against *P. expansum* inoculated on "d'Anjou" pears. Those researchers stated that this treatment was not effective in controlling decay caused by this fungus but, at the same time, lesion diameters in the ozone-treated fruit were significantly smaller than those in the control fruit.

When the pears were immersed in water with ozone concentrations of 4.2 to 5.5 μg/mL, 81–100% of the wounds became infected regardless of the exposure time from half to five minutes and with no differences in lesion size with respect to the control group. However, the germination of spores of *B. cinerea, M. piriformis*, and *P. expansum* was inhibited by treatment in ozonated water (0.1–4 μg O_3/mL) and this could be used as an indirect mode of decay control by lowering the concentration of viable fungal propagules (Spotts and Cervantes, 1992).

In the evaluations performed during five months of storage at -1°C, these authors did not observe differences in decay levels from fruit floated for five minutes in chlorinated (55 μg/mL) and ozonated (0.31 μg O_3/mL) dump solutions in the packinghouse. Considering the $LD_{99–100}$ of chlorine (approx. 50 μg/mL) and O_3 for spores of *B. cinerea*, *M. piriformis*, and *P. expansum* (1.9, 1.4, and 0.8 μg/mL, respectively), the authors attributed the lack of effect of the latter to a low concentration of the gas in comparison with the chlorine.

In another set of experiments, the ethylene level in both apple and pear storage rooms was reduced by 0.4 μL/L ozone while the opposite occurred in the control rooms (Skog and Chu, 2001). These researchers reported that after 107 days of storage at 0°C and one week at 20°C, all the quality parameters studied (internal ethylene concentration, firmness, TSS, titratable acidity, and scald index) were similar in the control and the O_3-treated apple and pear samples.

In Table 2, a summary of the studies on ozone effects on microbial, physicochemical, and sensorial quality of pome fruits is presented.

Stone Fruits

Peaches

Several studies were conducted to investigate the effects of O_3 application on peaches. As with other fruits, the reported results are somehow contradictory and can be partially attributed to differences in the evaluation date. Ridley and Sims (1967) examined the peaches immediately after the O_3 treatment, while Spalding (1968) and Palou et al. (2002) analyzed the fruit after it was stored for 7 and 28 d, respectively. Exposure of peaches inoculated with spores of *M. fructicola* or *Rhizopus* sp. to 0.5

Table 2 Ozone treatment studies on pome fruits

Fruit	O_3 Treatment	Parameters studied	References
Apples	0.006 ppm O_3 **gas**—4 h/d	Spoilage, weight loss	Bazarova, 1982
	2 to 10 cm^3 **gaseous** O_3/m^3 of air "some few hours" a day	Shelf-life extension	Kuprianoff, 1953
	1.95 ppm **gaseous** O_3—1 to 2 h daily—5 months	Fruit decay and rotting Fungal spore counts	Schomer and McColloch, 1948
	3.25 ppm **gaseous** O_3—8 h/d—7 months	Physiological damage Scald incidence Surface molds on packages and walls of apple storage rooms	
	0.4 to 4 ppm **gaseous** O_3—continuous or intermittent	Off-odors elimination Ripening delay Spore counts and germination Scald incidence	Smock and Watson, 1941
	3 ppm **aqueous** O_3—5 min	*E. coli* O157:H7 and *L. monocytogenes* inhibition Spoilage microflora growth Sensory quality	Rodgers et al., 2004
	25 mg/L **aqueous** O_3 - 4, 22 and 45°C—1, 3, and 5 min treatment	*E. coli* O157:H7 growth on inoculated apples	Achen and Yousef, 2001
Pears	0.4 μL/L O_3 **gas** in the storage room	Ethylene production Physicochemical parameters Scald index	Skog and Chu, 2001
	Line spray of **ozonated water**—3.1 μg O_3/mL, about 5 sec or immersion in water with 4.2 or 5.5 μg/mL O_3—0.5 to 5 min	*P. expansum* incidence and severity on inoculated d'Anjou pears	Spotts and Cervantes, 1992
	Ozonated water—0.1 to 4 μg O_3/mL—1, 3, and 5 min	*B. cinerea, M. piriformis* and *P. expansum* spore germination	
	0.31 μg/mL **aqueous** O_3 - 5 min	Fungal propagules concentration in dump water Pear decay during 5-month storage	

and 1 ppm of O_3 for 24 hours was effective in reducing the percentage of rotted fruit in comparison with the untreated control (Ridley and Sims, 1967). In these experiments, the higher the gas concentration, the larger the percentage of sound fruit. Both concentrations of O_3 reduced also the percentage of surface area of fruit affected by rot (Ridley and Sims, 1966). In contrast, the percentage of inoculated peaches of three cultivars that developed *Rhizopus* and *Monilinia fructicola* disease after seven days of cold storage was not reduced by treatment with 0.5 ppm O_3 (Spalding, 1968), but ozone reduced the nesting of both fungi during one week at 15°C.

Palou et al. (2002) reported that a continuous exposure of "Elegant Lady" peaches wound inoculated with *Monilinia fructicola*, *Botrytis cinerea*, *Mucor piriformis*, or *Penicillium expansum* to 0.3 ppm (v/v) O_3 in the storage atmosphere for four weeks at 5°C and 90% relative humidity (RH) affected the external mycelial growth and sporulation of all the pathogens tested. However, no significant differences were observed between O_3 and control treatments for either incidence or decay caused by these fungi, with the exception of brown rot caused by *M. fructicola* (Palou et al., 2002).

In peaches of the cultivar "Zee lady," these authors found that the continuous exposure to 0.3 ppm of ozone increased water loss only after five weeks of storage at 5°C and 90% RH. No phytotoxic injuries on the fruit tissues were observed with O_3 concentrations up to 0.5 ppm, but with higher concentrations of the gas, brown sunken areas appeared at the stomata of the peaches (Spalding, 1968). On the other hand, there were no significant differences in firmness, CO_2 evolution, ethylene production, pH, soluble solids, total titratable acidity, and color as influenced by the concentration of O_3 (Ridley and Sims, 1967; Palou et al., 2002).

Sweet Cherries

Sweet cherries cv. "0900 Zirat" were precooled for 16 minutes with ozonated water (Koyuncu et al., 2008). These researchers stated that the treatment did not affect either the color, firmness, SSC, TA or the external appearance of the fruit. At the same time, the treated cherries maintained a greener color of the stems and a better marketability than the control samples during a seven-day storage period. Even when O_3 did not reduce the bacterial counts on the fruits, from the fifth day, a 1–2 log reduction was recorded for yeasts and molds with respect to the control cherries (Koyuncu et al., 2008).

Citrus

Clementines

"Nules" clementines inoculated with spores of *B. cinerea* were stored for eight days at 13°C and 95% RH in an atmosphere enriched with 0.1 μmol/mol ozone. Both, lesion development and spore production were significantly reduced by the O_3 treatment in comparison with air-stored fruit (Tzortzakis et al., 2007b). These are unexpected results as a very low O_3 concentration was used together with a relatively high temperature, which, in turn, would enhance a faster O_3 decomposition into O_2.

Lemons

Palou et al. (2001) exposed inoculated "Eureka" lemons to 0.3 ppm O_3 applied in a day–night cycle during three weeks of storage at 4.5°C. These authors found that the incidence of blue mold was delayed, but not reduced, by the O_3 treatment during this period. However, after seven weeks of storage, incidence was 100% for both the control and the ozonated samples. In contrast, sporulation was greatly reduced during all the storage period and none of the fruit appeared injured by the ozone treatment.

Oranges

Exposure to continuous 0.3 ppm O_3 delayed both green and blue mold incidence on inoculated "Valencia" oranges about one week with respect to ambient air (Palou et al., 2001). Additionally, infections developed more slowly and both, external mycelial growth and conidia development of *P. italicum* and *P. digitatum,* were prevented or reduced by the treatment with gaseous O_3. Similar results were described by Di Renzo et al. (2005) with "Ovale" and "Valencia" oranges stored for eight weeks at 5°C and 90–95% RH. Before storage, the fruits were washed in chlorinated (50 mg/L of chlorine) or ozonated water (0.6 mg O_3/L), and these treatments were combined with 0.25 ppm of ozone concentration in the storage atmosphere during the night. These authors reported that ozone also decreased the load of spores in the storage room and inhibited the surface growth of mold on packages, walls and floors. However, gaseous O_3 was not effective either in reducing the final incidence and severity of the molds after four weeks of storage at 5°C in the first case (Palou et al., 2001) or in reducing *Penicillium* incidence in unwashed fruits in the combined treatment (Di Renzo et al., 2005).

In another study, Palou et al. (2003) studied O_3 penetration and effectiveness when applied in different type of packages. No spores of either *P. digitatum* or *P. italicum* were present on inoculated "Lanelate" oranges after exposure to 0.72 ppm (v/v) ozone for 14 days when the fruit was packaged naked in vented containers with an acceptable percentage of ozone penetration. In contrast, 5–30% and 31–60% of the control fruit surface was covered with spores of *P. italicum* and *P. digitatum*, respectively (Palou et al., 2003). In this study, the importance of using highly vented packages and the need to guarantee a good circulation and distribution of the ozone in the storage rooms to obtain positive results in the treatment of fresh produce were highlighted.

On the other hand, Kuprianoff (1953) performed a set of experiments using up to 40 ppm O_3 in the storage atmosphere of oranges and did not find symptoms of phytotoxicity during the

Table 3 Ozone treatment studies on citrus fruits

Fruit	O_3 Treatment	Parameters studied	References
Clementines	Enrichment of the atmosphere with 0.1 μmol/mol O_3 **gas**—8 d	*B. cinerea* growth and spore production	Tzortzakis et al., 2007b
Lemons	Intermittent 0.3 ppm O_3 **gas**—3 weeks of storage at 4.5°C	Blue mold incidence and spore production	Palou et al., 2001
Oranges	Continuous 0.3 ppm **gaseous** O_3	Green and blue mold incidence Rate of ethylene destruction	Palou et al., 2001
	0.72 ppm O_3 **gas** (v/v)—14 d	O_3 penetration and effectiveness when applied in different type of packages to control *P. digitatum* and *P. italicum* spores growth	Palou et al., 2003
	Up to 40 ppm **gaseous** O_3 in the storage atmosphere	Ethylene reduction Physiological damage Ripening delay	Kuprianoff, 1953
	Ozonated water (0.6 mg O_3/L) + 0.25 ppm **gaseous** O_3 in the storage room	Fungal growth Spores load and surface growth of mold on packages, walls and floor	Di Renzo et al., 2005
Tangerines	Continuous exposure to 200 mg **gaseous** O_3/L for 2 h/day—28 d	*Penicillium digitatum* incidence Weight loss, TSS,TA, peel color	Whangchai et al., 2010

storage period. Other authors also reported that weight loss was similar in the fruits stored in the O_3 and normal air atmospheres (Di Renzo et al., 2005). Moreover, ozone was also effective in reducing the ethylene level within the containers, retarding the ripening process of the oranges with an enhanced rate of ethylene destruction with increased O_3 concentration (Kuprianoff, 1953; Palou et al., 2001).

Tangerines

Tangerines cv. "Sai Nam Pung" were inoculated with *Penicillium digitatum*, washed with electrolyzed water (EW) for 4, 8 and 16 minutes and stored 28 days at 5°C with continuous exposure to 200 mg ozone/L for two hours per day (Whangchai et al., 2010). The combined treatment of EW for 8 and 16 min + O_3 suppressed the disease incidence during 28 days of storage. On the contrary, disease incidence of the control and fruit washed for four minutes gradually increased after 14 days of storage. Moreover, the combined treatment was more effective in decreasing the disease symptoms than washing with EW alone and had no adverse effects on weight loss, total soluble solids, titratable acidity, or peel color of the fruit.

In Table 3, different research works dealing with ozone effects on microbial, physicochemical, and sensorial quality of citrus fruits are summarized.

Tropical Fruit

Bananas

It is worth noting that there are no recent research works dealing with O_3 effects on bananas and those already published are mainly focused on physiological parameters (respiration rate, ripening), with no references to microbiological or sensory quality mentioned in those reports.

Gane (1937) informed that O_3 concentrations in the range of 1.5 to 7 cm^3/m^3 of air did not affect either the respiratory rate or the ripening process, but produced physiological damage on the O_3-treated bananas with injuries in the peel of the fruit. When O_3 dosage was increased to the range of 25 to 90 cm^3/m^3 and after eight days, a higher respiration rate was observed together with blackening of the banana peel and a retard of the ripening process due to the destruction of ethylene (Kuprianoff, 1953). Finally, the exposure of ripening bananas to 70–90 ppm of ozone retarded the rate of ripening, but only if the fruit was not within a few days of its climacteric period. However, with these O_3 concentrations, physiological damage was observed on the fruits (Gane, 1937).

Kiwi

Barboni et al. (2010) tested an atmosphere enriched with 4 mg/h of gaseous O_3 as an alternative to air for a seven-month storage period of "Hayard" kiwi fruit kept at 0°C and 90–95% RH. Through the exposure to O_3 it was possible to control *B. cinerea* during all the storage period, while rot caused by this fungus was detected from week 21 in fruits stored in air. However, no reference to the equilibrium O_3 concentration reached in the treatment chambers is given in this work.

In another experiment, Minas et al. (2010) also reported a delay of about a month in the appearance of stem-end rot on inoculated kiwis stored in an O_3-enriched atmosphere (0.3 μL/L) with respect to the control stored in air. After a four-month storage period, the treatment with O_3 reduced disease incidence by 56% and also inhibited fungus sporulation whereas disease severity was not affected. What is more, when the kiwis were exposed to O_3 for up to 144 hours before inoculation, disease incidence was reduced and, at the same time, an accumulation of phenolic compounds was observed. According to these authors, this fact may indicate that O_3 might have induced a defense reaction in the kiwis against *Botrytis* attacks.

Concerning physicochemical attributes, O_3 did not affect the weight loss, firmness, °Brix, TA, pH, sugars, and vitamin C of the kiwis with respect to the control fruit, while the main

Table 4 Ozone treatment studies on tropical fruits

Fruit	O_3 Treatment	Parameters studied	References
	GASEOUS O_3		
Banana	1.5 to 90 cm^3 O_3/m^3 air	Ethylene destruction	Gane, 1937
		Physiological damage	
		Respiratory rate	
		Ripening process	
Kiwi	4 mg/h—7 months	*Botrytis cinerea* rot	Barboni et al., 2010
		Weight loss, firmness, °Brix, TA, pH, sugars and vitamin C	
	0.3 μL/L—4 months	Stem end rot incidence	Minas et al., 2010
		Phenolic compounds accumulation	
Longan	20 ppm—15 to 120 min	*Lasiodiplodia* sp. and *Cladosporium* sp. incidence	Whangchai et al., 2005
		Mold, yeast and bacteria inhibition	Whangchai et al., 2006
		Browning	
Persimmon	Continuous 0.15 ppm—30 d	Color index, TSS, pH	Salvador et al., 2006
		Ethanol and acetaldehyde	
		Softening	
		Weight loss, electrolyte leakage	

organic acids were better maintained in conventional storage at 0°C (Barboni et al., 2010).

Longan

Whangchai et al. (2005) investigated the efficacy of an exposure to 20 ppm gaseous ozone for 0 (control), 15, 30, 60, and 120 minutes to control *Lasiodiplodia* sp. and *Cladosporium* sp. inoculated on longan fruits. The authors concluded that the best results in reducing the population of both microorganisms and disease incidence percentage within six days of incubation at 28°C were obtained with an exposition to the gas of 60 minutes. When the time of exposure to ozone was increased to 120 minutes, only slight decreases in disease incidence were observed with respect to the control. These results could reflect damages to the fruit cuticle by the longer treatments, which, in turn, make the fruit more susceptible to decay. This fact, together with the high storage temperature used, could enhance the fungal growth. Moreover, no references to the temperature at which O_3 treatment was applied are given in this work while this is an important factor to be considered, as ozone degrades faster with higher temperatures.

In another set of experiments, an exposure of longan fruit cv. "Daw" to 20 ppm of gaseous ozone for 60 minutes was also effective in reducing mold, yeast, and bacteria populations on the fruit surface immediately after the treatment and for three days in storage at 25°C and 75% RH. Within 3 d, 61% of control fruits were infected, whereas only 37% of the ozone-treated fruit was affected (Whangchai et al., 2006). Conversely, when the exposure time was increased to 120 minutes, and as it was mentioned above, damages to the cellular membrane of the fruit were observed and disease incidence increased. Furthermore, when compared with the control group, the combination of O_3 treatment with a five-minute immersion in a solution containing 5% of citric or oxalic acid, not only reduced disease incidence but also diminished browning for over three weeks in storage at 5°C and without affecting the eating quality of the fruits (Whangchai et al., 2006). These authors concluded that O_3 alone or combined with either oxalic or citric acid could be, at least, a partial alternative to SO_2 fumigation to control postharvest decay and browning.

Persimmon

Salvador et al. (2006) stored persimmon fruits cv. "Rojo Brillante," picked at two different harvest dates, in air or air enriched with continuous 0.15 ppm of O_3 for 30 days at 15°C and 90% RH + 7 d at 20°C and 90% RH to simulate shelf-life. These researchers determined that O_3 did not affect (early harvest, more immature fruit) or even delayed (second harvest) softening of the fruit after the shelf-life simulation, maintaining in this case firmness over the commercial limits. However, O_3-treated persimmons showed higher weight loss and electrolyte leakage than the control fruits, especially after shelf-life, and this effect was related to cuticle damage caused by the exposure to the gas.

On the contrary, color index (CI), TSS, pH, and ethanol and acetaldehyde production were not significantly affected by the O_3 treatment. What is more, no symptoms of phytotoxicity were observed in the ozone-treated fruits.

In Table 4, a summary of the researches on the effects of ozone application on tropical fruits is presented.

Dried Fruits and Nuts

For both, dried figs and pistachios, better results were obtained with increasing gaseous O_3 concentrations. This could be explained by the low a_w of the dried fruits, as O_3 is less reactive with low water contents, and there is also a need for moisture in the cells in order to be inactivated by O_3.

Figs

The treatment of dried figs with 1, 5, and 10 ppm of gaseous O_3 reduced the total aerobic mesophilic, coliform, and yeast and mold counts on the fruits treated for three or five hours

at 20°C with respect to an untreated control. *E. coli* was not detected on any of the analyzed samples. Microbial counts of the three groups decreased with increasing O_3 concentration, while no effects of the exposure time were found except for the total aerobic mesophilic microorganisms (Öztekin et al., 2006). These researchers concluded that a minimum of three hours ozone treatment at 5 ppm could be successfully used to eliminate coliforms' colonies and to reduce aerobic mesophilic and yeasts and molds counts by 38 and 72%, respectively.

O_3 was also studied to reduce populations of pathogenic *E. coli*, *B. cereus*, and *B. cereus* spores. Inoculated dried figs were subjected to 0.1, 0.5, and 1 ppm of gaseous O_3 for 360 minutes at 20°C and 70% RH (Akbas and Ozdemir, 2008a). According to these authors, *E. coli* and *B. cereus* counts were diminished by 0.9–1.4 and 2.7–2.9 log units, respectively, with 0.1 and 0.5 ppm of the gas. Furthermore, when 1 ppm of O_3 was used, it was possible to achieve 3.5 log reductions for both bacteria.

However, higher concentrations of O_3 were necessary to significantly reduce *B. cereus* spores. According to Broadwater et al. (1973) the spore coat may act as a primary protective barrier against ozone. Treatment of dried figs for 360 minutes with 1, 5, 7, and 9 ppm O_3, resulted in decreases of *B. cereus* spores of 1, 1.5, and 2 log units, respectively, with no differences between 7 and 9 ppm. Regardless of O_3 concentration, the treatment with the gas did not affect the physicochemical (color, pH, and moisture content) and sensory (sweetness, rancidity, flavor, appearance, and overall palatability) quality of the dried figs (Akbas and Ozdemir, 2008a).

Pistachios

In the studies carried out by Akbas and Ozdemir (2006a), whole and shelled kernels and ground pistachios inoculated with *E. coli* and *B. cereus* were exposed to 0.1, 0.5, and 1 ppm of gaseous O_3 for various times (0 to 360 minutes) at 20°C and 70% RH. Those authors observed that the effectiveness of ozone against both bacteria improved with increasing exposure time and ozone concentration. With O_3 dosages <1 ppm the reductions achieved for *E. coli* were of 2-3 log units in whole and shelled kernels, respectively. At the same time, *B. cereus* populations were reduced in 1.5–2 log units in whole and shelled kernels, respectively.

By increasing O_3 concentration to 1 ppm reductions of 3.5 and 3 log units were possible to achieve for *E. coli* and *B. cereus*, respectively and with no differences between whole and shelled pistachios. When the pistachios were ground, the results were similar for both pathogens with reductions of 1.5 and 2 log units for the lower and higher O_3 concentrations, respectively.

Akbas and Ozdemir (2006b) reported similar results when O_3 was evaluated for its capacity to degrade aflatoxins. Exposures to 9 mg/L of gaseous ozone for 420 minutes were effective to degrade these toxic compounds, with greater efficacy in whole kernels than in ground nuts. This showed that surface area of the pistachio samples was a critical factor for the effectiveness of the ozone treatment.

The treatment with O_3 did not affect the pH, color, rancidity, free fatty acids content or fatty acid composition of the samples (Akbas and Ozdemir, 2006a). At the same time, the whole kernels maintained also their sensorial quality unchanged (Akbas and Ozdemir, 2006b). On the other hand, the peroxide value was increased and flavor, sweetness, appearance, and overall palatability on ground pistachios were slightly affected when the highest concentration of O_3 was used.

ROOT VEGETABLES

Bulbs, Tubers, and Roots

In general, for onions and potatoes, positive results were observed with exposures to relatively low concentrations of gaseous O_3 to control natural microflora and with no physiological damages. The presence of several dry leaves and the peel in the onion bulbs and potatoes, respectively, may have protected these produce from the deleterious effects O_3 may cause on treated products.

In contrast, carrots were more prone to develop physiological damage. This could be partially explained by the absence of a protective skin in the case of onions and potatoes. Also, surface discoloration symptoms could be caused by the oxidative action of the O_3 on the carotenoids present in these roots.

Onions

In their study with onions, Fan et al. (2001) found that the exposure of the bulbs to 50 ppb O_3 during the day and 250 ppb during the night reduced weight loss and mold incidence after four weeks of cold storage and an additional two weeks of shelf-life. Faitel'berg-Blank et al. (1979) reported similar results for onion bulbs treated with 0.2 μg O_3/L for eight hours per day on five days a week. These authors found that losses due to spoilage at the end of the storage period were of 1% in the onions exposed to O_3 versus 9.7% for the control bulbs. Furthermore, due to O_3 treatment a reduction in chemiluminescence, oxygen uptake, catalase, and peroxidase activities were observed.

O_3 was also effective in reducing the airborne spore concentration in the storage rooms and the surface discoloration without affecting the internal decay, firmness, sprouting, and rooting, which were similar in the O_3-treated and in the control bulbs. Furthermore, no signs of phytotoxicity were detected on onions as a result of the corona treatment (Song et al., 2000).

Potatoes

In early studies, Kolodyaznaya and Suponina, (1975) demostrated that microbial growth was inhibited and superficial pathogenic microflora was destroyed on potato tubers stored in atmospheres containing 10 to 20 mg/m^3 of ozone. Similar results were observed in potatoes stored for six months at 6–14°C and 93–97% RH in an atmosphere containing 3 mg O_3/L

(Baranovskaya et al., 1979). In these conditions, bacteria and mold counts were "very low" for O_3-treated samples and the shelf-life of the potatoes could be extended, with similar results for onions and sugar beets. As it was mentioned above for onions, exposure to 0.2 μg O_3/L for eight hours a day on five days per week during storage of potatoes reduced losses due to spoilage from 6.7% in the controls to 0.8% in the treated tubers (Faitel'berg-Blank et al., 1979).

More recently, Selma et al. (2006) carried out experiments to evaluate the effects of aqueous O_3 to inactivate *Y. enterocolitica* on inoculated potatoes. A 30-second exposure to ozonated water (5 ppm O_3) reduced the microbial population by 1.6 log units. No further cell inactivation was obtained by prolonging the O_3 treatment for up to five minutes. In the same study, it was observed that a 5-ppm ozonated water wash for one minute decreased mesophilic and psychrotrophic bacteria, coliforms, and *L. monocytogenes* counts by 1.1, 0.7, 1.5, and 0.8 log units on potatoes, respectively. The reductions in the microbial groups were not enhanced when the exposure time increased from one to seven minutes.

The effects of O_3 on the physicochemical quality of potatoes were also studied in several research works. After storage, the tubers stored in the O_3 atmospheres presented a 3 to 6% higher starch content, 1.3 to 1.5 times lower total sugar content and 1.2-fold higher vitamin C concentration than the controls, while respiration rate was only slightly affected (Kolodyaznaya and Suponina, 1975). On the other hand, Baranovskaya et al. (1979) did not observe changes in the chemical composition and sensory quality of potatoes after a six-month storage period. Finally, chemiluminescence, oxygen uptake, and catalase and peroxidase activities were also reduced by exposure to O_3 (Faitel'berg-Blank et al., 1979).

Carrots

Application of gaseous O_3 on carrots was studied mainly for its effects on *B. cinerea* and *S. sclerotiorum* during storage with both positive and negative results, which could be attributed to the physiological damage caused on the carrots, depending on the O_3 dose and the duration of the treatment.

Whole inoculated carrots with *B. cinerea* and *S. sclerotiorum* were exposed to O_3 concentrations ranging from 0 (control) to 60 μL/L for eight hours daily for 28 days and stored at 2, 8, and 16°C (Liew and Prange, 1994). Growth rates of both fungi showed a trend to decrease with increased O_3 concentration within each storage temperature. With the highest O_3 dose, it was possible to achieve a 50% reduction in the growth rate compared with the control treatments.

In another set of experiments, whole carrots were treated with 300 and 1000 nL/L of O_3 for one, two, and four days before storage at 10°C and high RH to study the effects of the gas on saprophytic molds growth (Song et al., 2003b) and on the resistance to *B. cinerea and S. sclerotiorum* (Forney et al., 2007).

The lowest O_3 concentration was effective in reducing mold incidence for about eight weeks after the treatment, while 1000 nL/L applied for either two or four days had no beneficial effects on controlling the incidence of mold, but rather enhanced its development (Song et al., 2003b). On the other hand, this treatment induced decay resistance to *B. cinerea,* which lasted for the 24-week storage period. In contrast, the natural resistance to this fungus in the control samples was lost during the first eight weeks of storage and resulted in a doubling of the mycelial growth in comparison with the O_3-treated carrots (Forney et al., 2007). On the other hand, exposure to O_3 was not effective in inducing resistance to *S. sclerotiorum.*

Likewise, continuous O_3 concentrations of 0.05 ppm (Hildebrand et al., 2008) and, 0.115 and 0.530 ppm (Hildebrand et al., 2001) were also tested against these fungi during cold storage. At 0.115 ppm of O_3, lesion expansion and mycelial height of both pathogens were reduced, while sclerotiums' formation was prevented. On the contrary, no further positive effects were found with the highest concentration and, at the same time, symptoms of phytotoxicity were developed (Hildebrand et al., 2001). The exposure to 0.05 ppm of O_3 did not completely control decay, but reduced the disease severity caused by both *B. cinerea* and *S. sclerotiorum* and the incidence of saprophytic molds in the roots, reducing at the same time the carrot-to-carrot mycelial spread of pathogens (Hildebrand et al., 2008). These authors concluded that the treatment with O_3 could be useful in reducing nesting under storage conditions.

When working with carrots inoculated with *P. carotovorum*, Hassenberg et al. (2008) determined that the bacteria population was reduced by 1.5 log units after washing for two minutes with water containing 4 ppm ozone. Conversely, the maximum reduction obtained by washing with tap water was of 0.5 log units. No further decreases in the number of bacteria were observed by extending the washing time for up to 10 minutes (Hassenberg et al., 2008). The high reactivity of ozone with any organic material in the wash water or contaminating carrot roots, may have contributed to the reduction of ozone efficiency.

Concerning physicochemical parameters, O_3 applied in either gaseous (Liew and Prange, 1994; Forney et al., 2007; Hildebrand et al., 2008) or aqueous (Hassenberg et al., 2008) phase did not affect carrot weight loss, sprouting, tuber conductance or vitamin C content. On the contrary, an increased respiration rate, ethylene production and electrolyte leakage were associated with exposures to O_3. The treatments with this gas affected also the intensity of color, mainly with increasing gas concentrations (from 0.3 to 60 ppm) and longer expositions extent (Liew and Prange, 1994; Song et al., 2003b). Though, other researchers did not detect the effects of O_3 treatments on either ethylene production or on respiration rate of stored carrots (Forney et al., 2007; Hassenberg et al., 2008).

Concentrations of 60 μL O_3/L applied for eight hours a day provoked physiological damage to the carrots. Injury symptoms included pitting of the carrot surfaces with dry white blotches and brown water-soaked lesions on carrot leaves (Liew and Prange, 1994). These authors concluded that an ozone supply

of 15 μL/L for eight hours a day at 2°C could provide disease protection with a minimum of physical and physiological damage.

Even when O_3 concentration was lowered to 300 or 1000 nL/L applied for one, two or four days, treated carrots showed a logarithmic increase of the concentration of 6-methyoxymellein (6-MM or isocumarin) with increasing O_3 dose (concentration $\times$ time) (Song et al., 2003b). Furthermore, when the treatment with O_3 lasted for four days, softening of the tissues, loss of sucrose levels, increase in glucose and fructose concentration, and stimulation of stress volatiles (ethanol, hexanal, hexanol, and trans-2-hexenol) production were observed, indicating significant physiological injury as a result of the oxidative stress (Forney et al., 2007).

During six months of storage in an atmosphere enriched with 50 nL/L of O_3, sprouting of carrot crowns and sugar concentration were not affected with respect to the control samples stored in air. On the other hand, O_3 induced 6-MM accumulation and caused the appearance of slight injury in the periderm (Hildebrand et al., 2008). According to these authors, despite this damage, this treatment could be useful commercially to reduce nesting caused by *B. cinerea* and *S. sclerotiorum* in carrots stored and destined for the processing market. In this case, any discoloration and most of the 6-MM, which accumulates mainly in the peel, would be removed during peeling.

A summary of the studies on ozone effects on quality of whole onions, potatoes, and carrots is presented in Table 5.

LEAFY VEGETABLES

Spinach

Vurma et al. (2009) proposed a combined treatment which could be easily adapted to the existing fresh produce processes.

Fresh baby spinach leaves were inoculated with *E. coli* O157:H7 and treated with O_3 during the vacuum cooling process. The following conditions were used for the exposure to the gas: O_3 applied at 935 ppm (vol/vol), 10 psig of holding pressure and 30-minute holding time combined with continuous 10 ppm of O_3 (vol/vol) for up to three days of simulated transportation (Vurma et al., 2009). A decrease of 1.8 log CFU/g in *E. coli* counts was observed immediately after the vacuum cooling, and this pathogen remained undetectable (>5-log reduction) after the combined treatment with no damages to the quality of the treated leaves.

FLOWER BUDS

Broccoli

Forney et al. (2003) showed that both, 200 and 700 nL/L of O_3 were effective in reducing or eliminating mold growth on the sepals of broccoli flower buds and delaying yellowing appearance of the florets during 12 days of storage at 12°C. Similar results were observed in broccoli stored with 40 nL/L O_3, at either 3 or 10°C. These florets presented less browning of the base and less yellowing and floret opening than the control samples (Skog and Chu, 2001).

On the other hand, the highest O_3 concentration (700 nL/L of O_3) was injurious with the main symptoms of the physiological damage being desiccation of the sepals, browning of the stem ends, reduction in chlorophyll fluorescence, altered volatile composition, and enhanced weight loss. CO_2 and ethylene production were increased for two and five days, respectively, but final values were similar to the control group. None of these symptoms were observed when 200 nL/L of O_3 were used (Forney et al., 2003).

FRUIT VEGETABLES

In general terms, treatments with both, aqueous and gaseous O_3, were effective in reducing either natural or inoculated microbial load and in keeping sensorial and nutritional quality of the O_3-treated fruits. To completely inactivate the microorganisms, higher O_3 concentrations together with longer exposures to the gas were needed when high doses of inoculum were used.

Cantaloupes

Rodgers et al. (2004) washed unwaxed cantaloupes ("Eastern" variety) inoculated with *E. coli* O157:H7 and *L. monocytogenes* with water containing 3 ppm of O_3 for five minutes. These researchers published that immediately after the treatment, *E. coli* O157:H7 and *L. monocytogenes* were no longer detected on the melons and remained undetectable during nine days of storage at 4°C. On the contrary, washing the melons with tap water reduced the pathogens populations by approximately 1 log and subsequent growth during nine days of storage did not exceed 1 log.

After the exposure to O_3, the reductions achieved for mesophilic bacteria, yeasts, and molds were of approximately 4 to 5, 1 and 2.5 log units, respectively. In contrast, washing with tap water was not effective against yeasts and reduced molds and mesophilic bacteria by 1 and 0.7 log units, respectively. During storage, all the microbial groups' populations increased with final counts higher than the initial values and, in a greater extent, in the control samples. Also, no significant differences were found in the sensory analyses between the O_3-treated and the control melons (Rodgers et al., 2004).

Cucumbers

The exposure of cucumbers to 0.04 μL/L of O_3 during 17 days of storage at 3°C was effective in maintaining firmness, a better appearance (based on chilling injury and amount of decay) and lower microbial counts than in the control group stored in air. On the other hand, yellowing was present in both the ozonated and control samples with no visual differences between them (Skog and Chu, 2001).

Table 5 Ozone treatment studies on whole onions, potatoes and carrots

Vegetable	O_3 Treatment	Parameters studied	References
Onions	50 and 250 ppb O_3 **gas** (day and night, respectively)	Firmness Mold incidence Sprouting Surface discoloration Weight loss	Fan et al., 2001
	0.2 μg **gaseous** O_3/L for 8h/d, 5 d/week	Catalase and peroxidase activities Chemiluminescence Oxygen uptake Spoilage	Faitel'berg-Blank et al., 1979
	50 and 250 ppb O_3 **gas** (day and night, respectively)	Airborne mold sampling Ethylene and volatile analysis Firmness Internal decay Roots and sprouts formation Surface discoloration Weight loss	Song et al., 2000
Potatoes	10 to 20 mg/m^3 of **gaseous** O_3	Microbial growth Respiration rate Starch, sugars and vitamin C Superficial pathogenic microflora	Kolodyaznaya and Suponina, 1975
	6 months at 6–14°C, 93–97% RH in an atmosphere with 3 mg **gaseous** O_3/L	Chemical composition of the tubers Microbial growth Sensory quality Shelf-life extension	Baranovskaya et al., 1979
	0.2 μg **gaseous** O_3/L for 8h/d, 5 d/week	Catalase and peroxidase activities Chemiluminescence Oxygen uptake Spoilage	Faitel'berg-Blank et al., 1979
	Aqueous O_3: 5 ppm—1–7 min	Mesophilic, psychrotrophic, coliforms and *L. monocytogenes* counts	Selma et al., 2006
	5 ppm −30 s to 5 min	*Y. enterocolitica* inactivation	
Carrots	**Gaseous** O_3: 0 to 60 μL/L for 8 h/d—28 days	*B. cinerea* and *S. sclerotiorum* growth rate Color changes Electrolyte leakage Respiration rate, weight loss	Liew and Prange, 1994
	300 and 1000 nL/L of O_3 **gas** at 10°C for 1, 2, and 4 days before storage at 0°C for 24 weeks	Decay and mold incidence Electrolyte leakage Surface discoloration, 6-methyoxymellein content	Song et al., 2003b
		Aroma and stress volatiles Firmness, weight loss Physiological damage Resistance to *B. cinerea and S. sclerotiorum* Respiration rate and ethylene production Sugar content	Forney et al., 2007
	Continuous O_3 **gas**: 115, 208, and 530 ppb	Resistance to *B. cinerea and S. sclerotiorum*	Hildebrand et al., 2001
	Continuous 50 nL/L O_3 **gas**—6 months of storage at 0.5°C and > 95% RH	Resistance to *B. cinerea and S. sclerotiorum* Saprophytic molds growth Sprouting Sugar concentration, 6-MM accumulation Weight loss	Hildebrand et al., 2008
	Aqueous O_3: 4 ppm—2–10 min	*P. carotovorum* control Respiration rate Tuber conductance Vitamin C content	Hassenberg et al., 2008

Tomatoes

In early studies, spores of *B. cinerea* on the surface of non-injured tomato fruits were found to be inactivated when exposed to 3.8 μg/mL ozone solutions for 10 minutes (Ogawa et al., 1990). However, according to these authors, the spores placed in surface injuries were not inactivated by this treatment. In contrast, *B. cinerea, A. alternata*, and *C. coccodes* spore production was inhibited by 94–99, 55–80, and 13–74%, respectively, in wound inoculated "Mareta" tomatoes maintained

in ozone-enriched atmospheres (0.05 to 5 μmol/mol) for 2 to 312 hours, compared with control fruit stored in air (Tzortzakis et al., 2007b, 2008). Moreover, these authors found that lesion development in the tomatoes exposed to O_3 prior to, or following inoculation with these fungi, was significantly reduced with respect to the control. Greater reductions were observed with higher concentrations of the gas and longer exposure times. What is more, no visible symptoms of injury were detected, even at the highest ozone concentration employed.

O_3 efficacy was also evaluated to reduce bacteria contamination. Cells of *S. enteritidis* inoculated on cherry tomatoes died completely after an O_3 gas treatment of 10 mg/L applied for five minutes for low-dose inoculum. For high-dose inoculum, 15 and 20 minutes were necessary to completely inactivate the pathogen for the one- and four-hour attachment times, respectively (Daş et al., 2006). In this study, no effective results were obtained with 5 mg O_3/L, while with 20 mg/L a complete reduction of the pathogen population was observed after 15 minutes for four-hour attachment time.

Related to the effects of O_3 treatments on physicochemical and nutritional quality, in general, fruits respiration rate, transpiration, ethylene emission, weight loss, color, TA, SSC, sugars, organic acids, and vitamin C contents were not affected, when compared to the air-stored samples, by either continuous (Tzortzakis et al., 2007a; Rodoni et al., 2010) or cyclic (Aguayo et al., 2006) exposures to O_3.

Neither antioxidant status, β-carotene, lutein, nor lycopene content were affected by the treatment with 0.05 to 1 μmol/mol of O_3 (Tzortzakis et al., 2007a). These authors did not detect effects on the total phenolic compounds content during and/or following storage for up to six days in an O_3-enriched atmosphere. In contrast, exposure of tomatoes to 10 μL/L O_3 for 10 minutes resulted in higher concentrations of phenolic compounds (Rodoni et al., 2010). These differences could be attributed to the different concentrations of the gas used in both studies.

Softening was not affected (Daş et al., 2006), or it was reduced by O_3. In this sense, Rodoni et al. (2010) observed decreases in the pectin methyl esterase (PME) activity, pectin solubilization, and depolymerization after exposure to O_3 while, total cell-wall composition, hemicellulose content, and the β-galactosidase (β-Gal) and poligalacturonase (PG) activities of the tomatoes were not affected. These researchers affirmed that the lower disassembly of cell-wall polyuronides might be an important contributor to the reduced softening and damage of O_3-treated fruit.

In the sensory evaluations, after 15 days of storage, the O_3-treated tomatoes (cyclic exposure to 4 ± 0.5 μL/L of gaseous O_3 30 minutes every three hour) kept a good appearance and overall quality, while in control fruit these parameters fell beneath the limit of marketability (Aguayo et al., 2006). Moreover, 70% of the taste panelists expressing a preference chose fruit subjected to low-level ozone enrichment (Tzortzakis et al., 2007a).

The studies dealing with ozone application effects on fruit vegetables' quality are summarized in Table 6.

MINIMALLY PROCESSED FRUIT AND VEGETABLES

For minimally processed fruit and vegetables, O_3 was studied mainly applied in aqueous phase during the washing step. In this way, the disinfection with O_3 can be easily integrated in the production chain of these products. In general terms, positive results were reported, especially when O_3 was used in combined treatments. Anyway, concentrations and application times should be carefully chosen in order to avoid damages such as surface discoloration or browning in sensitive products like lettuce, broccoli, and spinach.

Potato Strips

Beltrán et al. (2005b) studied the effects of washing potato (cv. "Monalisa") strips with ozonated water (20 mg/L min) combined with storage for up to 14 days in MAP or under vacuum at 4°C. These authors stated that respiration rate was not affected by the O_3 treatment and browning was controlled by the combination of dipping in ozonated water plus vacuum storage. Furthermore, in these conditions, the potatoes maintained their typical aroma and a very firm and turgid texture during the 14 days of storage. Conversely, O_3 applied alone was not effective in reducing the total microbial population but, when combined with 300 mg/L of peroxyacetic acid, it was the most effective treatment in controlling microbial growth and in maintaining sensory quality. In effect, reductions of 3.29, 3.0, and 1.2 log units for the lactic, coliforms, and anaerobic bacteria, respectively, were possible to achieve with the combined treatment and in relation to water wash (Beltrán et al., 2005b).

Carrots

Baby carrots inoculated with *E. coli* O157:H7 were washed in water with 5.2, 9.7, and 16.5 mg O_3/L for 1, 5, 10, and 15 minutes (Singh et al., 2002). Compared to one or five minutes and, within each ozone concentration, these authors found significantly higher reductions in the microbial counts of the carrots washed for 10 minutes. Under these conditions, *E. coli* was reduced by 1.34, 1.68, and 1.80 log units with 5.2, 9.7, and 16.5 mg O_3/L, respectively, and no further reductions were achieved by increasing the washing time up to 15 minutes (Singh et al., 2002). According to these researchers, when ozone was applied in gaseous phase (2.1, 5.2 and 7.6 mg/L for 5, 10, and 15 minutes), *E. coli* O157:H7 was inactivated by 1.11 to 2.64 log cfu/g, with respect to the control carrots. In this case, improved bactericidal effects were observed with higher O_3 concentrations and longer exposures to the gas.

In another study, peeled and topped uncut carrots were washed with ozonated water (1.3 mg O_3/L, 120 s) before shredding. Gas evolution, color, texture, sugar content, sensorial parameters (Klaiber et al., 2004), phenylalanine ammonia-lyase (PAL) activity and phenolic contents (Klaiber et al., 2005) of the shredded carrots were not affected by the prewashing treatment. On the other hand, O_3 was not effective in reducing the

Table 6 Ozone treatment studies on fruit vegetables

Vegetable	O_3 Treatment	Parameters studied	References
Cantaloupes	**Aqueous** O_3: 3 ppm—5 min	*E. coli* O157:H7 and *L monocytogenes* inhibition Microbial growth Sensory analysis	Rodgers et al., 2004
Cucumbers	0.04 μL/L of O_3 **gas**—17 d of storage at 3°C	Appearance Firmness Microbial counts Yellowing	Skog and Chu, 2001
Tomatoes	**Gaseous** O_3: 0.05 to 5 μmol/mol for 2 to 312 h	*B. cinerea, A. alternata* and *C. coccodes* spore production	Tzortzakis et al., 2007b, 2008
	Gaseous O_3: 5–20 mg/L; 5 to 20 min	*S. enteritidis* inhibition Softening	Daş et al., 2006
	Gaseous O_3: 4 ± 0.5 μL/L, 30 min every 3 h	Color, weight loss Respiration rate Ethylene emission TA, SSC, organic acids Vitamin C content Sensory evaluation	Aguayo et al., 2006
	Gaseous O_3: 0.05 to 1 μmol/mol for 1 to 6 d	Antioxidant status β-carotene, lutein and lycopene contents Color, firmness Phenolic compounds Respiration rate Ethylene emission Sensory analysis TA, SSC, organic acids Vitamin C content Weight loss	Tzortzakis et al., 2007a
	Gaseous O_3: 10 μL/L—10 min	β-Gal, PG and PME activities Color Pectin solubilization and depolymerization Respiration rate Ethylene emission TA, SSC, organic acids Vitamin C content Total cell wall composition Hemicellulose content Weight loss	Rodoni et al., 2010
	Aqueous O_3: 3.8 μg/mL—10 min	*B. cinerea* spores inactivation	Ogawa et al., 1990

total aerobic, lactic and enterobacteria populations of the carrots when compared with the results obtained by washing with tap water. These effects were attributed to the short contact time of the produce with the ozonated water (Klaiber et al., 2004).

Celery

Zhang et al. (2005) evaluated the effects of washing fresh-cut celery with ozonated water (0.03, 0.08, and 0.18 ppm) for five minutes. The treatment was found to be effective in significantly reducing the total bacterial load both, immediately after the treatment and during storage at 4°C. Those authors observed that the highest O_3 dose yielded the maximum initial reduction in bacterial counts, of 1.69 log CFU/g. After nine days, the microbial population of all the O_3-treated samples was lower than in the control samples, with differences of approximately 1 log unit. In this study, the exposure to O_3 decreased the polyphenoloxidase (PPO) activity and respiration rate of the fresh-cut celery with greater extent of inhibition with increased O_3 concentration. Total sugar content was not affected by O_3, while vitamin C was higher in the ozonated samples when compared with the control. Moreover, the authors concluded that washing with 0.18 ppm O_3 was the most effective treatment in maintaining sensorial quality during storage.

Green Asparagus

An et al. (2007) used water containing 1 mg O_3/L to wash fresh-cut green asparagus for 30 minutes. These authors reported that by means of this treatment, it was possible to slow down the rate of lignin, cellulose, and hemicellulose contents accumulation during 25 days of storage in MA at 3°C, when compared with the control group. In addition, the O_3-treated spears stored in MAP, presented higher and lower antioxidant enzyme (superoxide dismutase, ascorbate peroxidase, and glutathione reductase) and PAL activities, respectively, than the control samples.

Baby Leaf Brassica Leaves

In comparison with cold tap water and heat shock treatment, the use of ozonated water (10 mg/L) for one minute with or without activation with UV-C effectively reduced the total mesophilic and naturally occurring *Listeria* spp. counts of four *Brassica* leaves without negatively affecting the respiration rate of the treated produce during eight days of cold storage (Martínez-Sánchez et al., 2008). Moreover, O_3 alone reduced the yeasts and mold counts on mizuna (*Brassica rapa* L. ssp. *nipposinica*) and watercress (*Nasturtium officinale*). On the other hand, these microbial groups were not affected on salad (*Eruca vesicaria*) and wild rocket (*Diplotaxis tenuifolia*) leaves, either by O_3 alone, water wash, or heat-shock treatment.

Cabbage

Youm et al. (2004) found a reduction in PPO activity and in the total bacteria, *E. coli*, yeast, and mold counts of minimally processed cabbage dipped in a solution of 3 ppm O_3. This effect was observed immediately after the treatment and was reported to last for three days in cold storage. However, from day 4, the authors noted similar counts in the control and the O_3-treated samples. It should be noted that no reference either to the magnitude of the microbial reductions or to the extent of the dipping treatment was mentioned in this article.

Cilantro

Wang et al. (2004) evaluated the efficacy of aqueous O_3 to extend the shelf-life of fresh-cut cilantro. From the results of their researches, these authors informed that both, initially and during four days of storage at 0°C, the washing treatment for five minutes with ozonated water and a sequential wash of five minutes with ozonated water plus five minutes with acidic electrolyzed water (AEW) lowered the total aerobic and *Enterobacteriaceae* counts with respect to the control samples. However, only the sequential washing (O_3 + AEW) yielded less counts than the control group during a storage period of 14 d. On the other hand, the O_3 alone was the most effective treatment to preserve the typical aroma and overall quality of the fresh-cut cilantro leaves, maintaining a green and fresh appearance. Furthermore, no symptoms of yellowing or dehydration and no traces of off-odor were detected. Nevertheless, it should be remarked that no reference was mentioned in the article concerning the O_3 concentration used for the study.

Rocket Leaves

Martínez-Sánchez et al. (2006) also studied the effects of aqueous O_3 on rocket leaves (*D. tenuifolia* (L.)DC.). After washing for one minute with ozonated water (10 mg/L), mesophilic and psychrophilic bacterial counts were reduced by about 1 log unit with respect to samples washed with tap water. The results from this study showed that during the shelf-life period, O_3-treated samples maintained lower mesophilic counts than the control for 8 and 12 days for samples stored in MA and air, respectively. In the case of psychrophilic bacteria, the microbial counts on O_3-washed leaves remained lower than the control for 15 and 12 days in the samples stored in MA and air, respectively.

On the contrary, O_3 was not effective in reducing the initial coliform counts by more than 0.5 log units when compared to water and yeasts and molds were effectively controlled only in the air-stored samples. Related to the physicochemical, sensory, and nutritional quality, those authors published that the treatment with O_3 did not affect either the gas composition in the MA packages, the initial visual quality, color, weight loss, texture, chlorophyll content, freshness or the polyphenol, and glucosinolate concentration of the rocket leaves. However, total vitamin C content of the samples after eight days of storage was significantly reduced by the treatment, especially in the leaves stored in MAP.

Spinach

Fresh-cut spinach leaves were immersed for three minutes in ozonated water (5 ppm) at room temperature and compared with unwashed samples (Rahman et al., 2010). When compared with the unwashed control, the exposure to O_3 was effective in significantly reducing total bacterial and yeasts and molds counts by 1.07 and 0.88 log units, respectively. This treatment also reduced the populations of *E. coli* O157:H7 and *L. monocytogenes* by 1.22 and 1.4 log units, respectively, in inoculated leaves.

In another set of experiments, Klockow and Keener (2009) treated packaged spinach leaves inoculated with *E. coli* O157:H7 with gaseous ozone and stored the produce for up to 24 hours. After a five-minute treatment, O_3 concentration reached 1.6 and 4.3 mg/L when air and O_2 were used as feeding gas, respectively. The treatment was effective in reducing the pathogen populations, achieving maximum reductions of 3–5 log CFU/leaf after 24 hours. However, after this period of storage, the O_3-treated leaves showed color degradation.

In Table 7, a summary of the researches on ozone effects on microbial, physicochemical, and sensorial quality of minimally processed leaf vegetables is presented, whereas due to the great number of research works on lettuce, this product was included in a separate table.

Lettuce

As it was previously mentioned for table grapes, the most studied O_3 applications on vegetables are those on fresh-cut lettuce and, mainly, on the iceberg type. These studies include research on the effects of O_3 on pathogenic and spoilage microflora, physicochemical, nutritional, and sensory quality of the lettuce.

In both, romaine (Singh et al., 2002) and iceberg (Yuk et al., 2006) inoculated lettuce, washing with 3 and 5 mg/L ozonated water reduced *E. coli* O157:H7 counts by approximately 1 log unit, regardless of the time of exposure (0.5 to 15 minutes). Increasing the O_3 doses to 9.7 or 16.5 mg/L, yielded a reduction in

Table 7 Ozone treatment studies on minimally processed leaf vegetables

Vegetable	O_3 Treatment	Parameters studied	References
Baby leaf	**Aqueous** O_3: 10 mg/L, 1 min with or without activation with UV-C light	*Listeria* spp. inhibition Mesophilic bacteria, yeasts and molds growth	Martínez-Sánchez et al., 2008
Brassica	**Aqueous** O_3: 3 ppm	*E. coli* inhibition Microbial counts PPO activity	Youm et al., 2004
Cabbage	**Aqueous** O_3: 5 min (a) (a) + 5 min acidic electrolyzed water	Appearance, aroma Aerobic and Enterobacterias inactivation Yellowing, dehydration	Wang et al., 2004
Cilantro	**Aqueous** O_3: 10 mg/L—1 min	Chlorophyll content Microbial analysis, O_2/CO_2 concentration Sensory analysis Vitamin C, polyphenol and glucosinolates contents	Martínez-Sánchez et al., 2006
Rocket leaves	In packaging **gaseous** O_3: 1.6 and 4.3 mg/L—5 min	*E. coli* O157:H7 inhibition Discoloration	Klockow and Keener, 2009
Spinach	**Aqueous** O_3: 5 ppm—3 min	*E. coli* O157:H7 and *L. monocytogenes* inhibition Total bacteria and yeasts and molds inhibition	Rahman et al., 2010

the pathogen populations of about 1.4 log units after 10-minute washing when compared with the control group. However, Singh et al. (2002) did not observe further reductions by prolonging the washing time to 15 minutes. These authors explained that the reduced efficacy of the ozonated water at low O_3 concentrations might be due to a high ozone demand of organic material in the medium. Conversely, the combination of one-minute exposure to 3 ppm O_3 plus 1% citric acid reduced the *E. coli* O157:H7 counts in 2.31 units but no residual antimicrobial effects of the combined treatment were observed after 10 days of storage at 15°C (Yuk et al., 2006).

It should be remarked that the storage temperature used was higher than the recommended temperatures for minimally processed produce and could enhance microbial growth, limiting the positive results observed immediately after the washing treatment.

When O_3 was applied in gaseous phase (2.1, 5.2, and 7.6 mg/L for 5, 10, and 15 minutes) *E. coli* O157:H7 was inactivated by 0.79 to 1.79 log cfu/g, with improved bactericidal effects with increased concentration and length of exposure. However, during the treatments of 10 or 15-minutes, discoloration of lettuce leaves was observed when the 5.2 and 7.6 mg/L O_3 concentrations were used (Singh et al., 2002).

Regarding *L. monocytogenes*, the treatment with O_3 alone was not effective in reducing the population of this pathogen even at the greatest concentration and exposure time (5 ppm O_3 applied for five minutes) (Yuk et al., 2006). On the other hand, the combined treatment with citric acid showed a greater efficacy with a reduction of 1.80 log units in the pathogen population. In another study, washing for two minutes with 2 ppm ozonated water was determined to be the optimum conditions for treatment of green leaf lettuce. Under these conditions, higher than 2-log reduction was observed in *L. monocytogenes* counts without affecting the overall visual quality of the lettuce (Ölmez and Akbas, 2009). These contradictory results could be explained by differences in the lettuce variety studied, the temperature of the water used (10 and 22 °C), the initial inoculum level, and the physiological state of the bacterial cells.

Lastly, Selma et al. (2007) evaluated the efficacy of O_3 to reduce *S. sonnei* population on shredded iceberg lettuce. These researchers observed a reduction of 0.7, 1.4, and 1.8 log units in *S. sonnei* counts after a five-minute washing with water containing 1, 2, and 5 ppm O_3, respectively. Moreover, results from this study showed that the effectiveness of the O_3 treatment was not enhanced by activating the ozonated water with UV-C light.

From early studies, Kim et al. (1999) concluded that bubbling ozone gas in water with rapid stirring was the most efficient system to deliver ozone for the inactivation of spoilage microorganisms in lettuce. Those researchers reported that bubbling ozone (1.3 mM) in a water-lettuce mixture for three minutes inactivated about 2 log of natural flora. The efficacy of the treatment was enhanced by extending the exposure time to five minutes, achieving decreases of 3.9 and 4.6 log in the populations of mesophilic and psychtrotrophic microorganisms, respectively.

Similar results were observed in subsequent researches. After immersion of fresh cut iceberg lettuce in ozonated water (10 and 20 mg/L, up to five minutes), the total mesophilic and coliforms populations were reduced by 1.6 and 3.2 log units, respectively, when compared with the control samples (Beltrán et al., 2005a). For both microbial groups, these differences were maintained during the storage period. Moreover, in the O_3-treated samples, the number of mesophiles was more than one $\log_{10}$ cycle below the recommended limits, while the control exceeded these values. Similar results were observed in trimmed heads and fresh-cut lettuce washed with cold ozonated water (Baur et al., 2004a; Hassenberg et al., 2007).

Other authors also found that dipping lettuce in ozonated water (4 mg/L) for two minutes reduced the initial load of mesophilic, psychrotrophic, and *Enterobacteriaceae* bacteria by 2.3, 2.2, and 1.8 $\log_{10}$ CFU/g, respectively. Ozone efficacy was similar to chlorine and organic acid solutions in reducing the mesophilic bacteria, while chlorine was the most effective

treatment against psychrotrophic bacteria (Akbas and Ölmez, 2007; Ölmez and Akbas, 2009). Likewise, 2.5 mg/L free available O_3 plus 100 mg/L free available chlorine resulted in the best combination to wash/rinse fresh-cut iceberg lettuce and to reduce the mesophiles and psycrothrophic microorganisms (García et al., 2003). These authors found that with this combination, it was possible to achieve a shelf-life of the lettuce greater than 25 days and, at the same time, improve the water quality in the processing line.

Opposite results were also reported (Koseki and Isobe, 2006; Ölmez and Akbas, 2009). After an initial reduction in the aerobic mesophilic and psychrotrophic bacteria population, the bacterial growth rate on lettuce washed with ozonated water increased during storage, yielding final counts similar to that of the samples washed with water alone.

Concerning the physicochemical, nutritional, and sensory quality, washing with ozonated water (1 to 20 mg/L O_3) did not affect the O_2 and CO_2 evolution, color, texture, moisture content, sensory attributes, phenolic compounds, β-carotene and vitamin C contents and PAL, PPO, and POD activities during cold storage of either trimmed heads or fresh-cut iceberg lettuce (Baur et al., 2004a, 2004b; Beltrán et al., 2005a; Akbas and Ölmez, 2007).

In contrast, when compared with calcium lactate, fresh-cut iceberg lettuce washed in water with 1 mg O_3/L presented a higher respiration rate and a significantly lower PPO and POD activity (Rico et al., 2006). Furthermore, the O_3-treated samples presented lower PME activity and lower values of crispiness coefficients, but these differences were not detected in the sensory evaluation.

A five-minute immersion in ozonated water (3, 5, and 10 ppm) did not affect the ascorbic acid content of fresh-cut iceberg lettuce, but provoked an increase in PAL activity and, with the highest dose, an increase in the a^* values compared with water washing alone (Koseki and Isobe, 2006). On the other hand, Hassenberg et al. (2007) reported that losses in vitamin C and sugar content could be reduced by washing with cold ozonated water with O_3 concentrations of 4–5 ppm compared with lettuce washed with tap water.

Regarding sensory quality, concentrations above 2.5 ppm O_3 in the washing water or exposure times longer than two and a half minutes enhanced losses in color and freshness together with appearance of browning (Ölmez and Akbas, 2009). These authors concluded that 2-ppm ozonated water applied for two minutes were the optimum conditions for washing green leaf lettuce. Under these conditions and after nine days of storage, the O_3-treated samples received higher scores in all the sensory parameters (overall visual quality, aroma, firmness, and browning inhibition) than the control ones washed in water alone.

A summary of the studies on ozone effects on quality of minimally processed lettuce is presented in Table 8.

Broccoli

Treatment of broccoli florets with water with 1 ppm O_3 for 10 or 50 minutes inhibited microbial growth over four days of storage at 5°C. However, after eight days microbial counts were similar to those observed in the control (Zhuang et al., 1996). In contrast, Das and Kim (2010) reported that the exposure of broccoli to water with 2 μL/L of O_3 for three minutes yielded the lowest counts of total aerobic and coliform bacteria during nine days of storage when compared with either tap, chlorinated or electrolyzed water.

Concerning the physicochemical quality, O_3 was found to accelerate surface discoloration and ascorbic acid loss of the treated florets by the end of the storage period (Zhuang et al., 1996). Conversely, these authors did not observe significant effects of the gas on either the total carotenoid and protein contents or the lipid peroxidation of the buds. On the other hand, in the study carried out by Das and Kim (2010), gas composition and color were not affected by the O_3 and no off-odors were detected at any time during the nine days of storage. These apparently contradictory results could be explained by the different duration of the exposure to O_3 in both experiments. In any case, an immersion of 10 to 50 minutes seems to be rather long for broccoli florets and excessive to be of industrial interest.

Cauliflower

Sothornvit (2010) treated fresh-cut cauliflower with ozonated water (0.31–0.35 ppm O_3, 15 minutes) and noted a reduction in the total plate and *E. coli* counts of 1.8 and 1.88 log units, respectively, compared with an unwashed control. Furthermore, these reductions were found to be higher than those achieved with either tap or chlorinated water and were maintained during 18 days of cold storage. Additionally, those authors stated that the O_3-washed cauliflowers maintained their white appearance, with the highest score in color during storage. No off-flavors were developed and the acceptability for consumers was 18 days, in contrast with 15 days for samples washed with tap water or unwashed ones.

Cantaloupes

The treatment of whole cantaloupe melons with gaseous O_3 (10,000 ppm—30 minutes), alone and combined with hot water (75°C—1 minute), was effective in reducing the microbial counts on the fruits, without affecting the subsequent global sensory quality of the fresh-cut melon after eight days of storage at 5°C (Selma et al., 2008a). Compared to the control samples, the O_3-treated melons showed reductions in the total mesophilic, psychrotrophic, molds, and total coliforms populations of 1.1, 1.3, 1.5, and 1.3 log units, respectively. Moreover, those researchers stated that the combined treatment was more effective than the O_3 alone. In this case, the microbial reductions were enhanced to 3.8, 5.1, 2.2, and 2.3 log units for the total mesophilic, psychrotrophic, molds, and total coliforms, respectively. Full typical aroma, color, and a very firm and turgid texture were maintained all over the storage period with no off-odors detected in the sensory evaluation. Even when the used O_3 concentration was very high and the duration of the treatment

Table 8 Ozone treatment studies on minimally processed lettuce

O_3 Treatment	Parameters studied	References
O_3 **gas**: 2.1, 5.2, and 7.6 mg/L; 5 to 15 min.	*E. coli* O157:H7 inhibition	Singh et al., 2002
AQUEOUS O_3		
5.2, 9.7, and 16.5 mg/L—1 to 15 min	*E. coli* O157:H7 inhibition	Singh et al., 2002
1, 3, and 5 ppm—0.5 to 5 min	*E. coli* O157:H7 and *L. monocytogenes* inhibition	Yuk et al., 2006
3 ppm O_3 + 1% organic acid −1 min	*L. monocytogenes* inhibition	Ölmez and Akbas, 2009
0.5, 2.5, and 4.5 ppm—0.5, 2, and 3.5 min	Natural microbial population counts Nutritional and sensory quality Wastewater quality	
1, 2, and 5 ppm O_3 2 ppm + UV-C activation - 0–5 min	*S. sonnei* population reduction	Selma et al., 2007
3–10 ppm; Sonication, stirring or stomaching for O_3 delivery	Spoilage microorganisms inhibition	Kim et al., 1999
10 and 20 mg/L—10 mg/L activated by UV-C light	Microbial analysis Phenolic compounds and vit. C Respiration rate Sensory quality	Beltrán et al., 2005a
1 mg/L. Washing trimmed heads prior to shredding—120 s at 4°C	Color, texture Microbial analysis O_2/CO_2 concentration Phenolic metabolism Sensory evaluation	Baur et al., 2004a, 2004b
3.6 ppm—water at 4–6°C	Aerobic mesophiles, *E. coli* and *Salmonella* spp. inhibition pH, TOC concentration in the process water Vitamin C and sugar analyses	Hassenberg et al., 2007
4 mg/L—2 min	Microbiological analysis Color, texture and moisture content, O_2/CO_2 concentration Vitamin C and β-carotene	Akbas and Ölmez, 2007
2.5, 5, and 7.5 mg/L—10 min. Alone and combined with chlorine	Microbiological analysis Sensory analysis Turbidity analysis in the water	García et al., 2003
3, 5, and 10 ppm—5 min	Browning Microbiological analysis PAL activity	Koseki and Isobe, 2006
1 mg/L—1 min. Alone and combined with 15 g/L calcium lactate—1 min	Browning and texture enzymes, Color, texture O_2/CO_2 concentration Sensory analysis	Rico et al., 2006

appears to be rather long, the skin of the melons could act as a protective barrier, avoiding damages to the flesh.

Peppers

Treatments with aqueous O_3 were not effective in reducing microbiological counts in either green or red peppers. This lack of effectiveness of the aqueous O_3 was attributed to the leaching of intercellular juices from the cut surfaces into the water, the migration of the microorganisms into the peppers or the fact that they become protectively attached to the cut surfaces of the peppers, suggesting that whole fruits could be more suitable for the treatment (Ketteringham et al., 2006). These researchers treated fresh-cut green peppers by immersion in ozonated water (0.30 and 3.9 mg O_3/L of water) for 20 seconds to 30 minutes, obtaining an O_3 effectiveness to reduce the aerobic plate counts similar to washing with water alone for 15 minutes. Similar results were obtained with fresh-cut red bell peppers washed with 1 ppm ozonated water for one, three, and five minutes (Horvitz and Cantalejo, 2010a). In this study, no positive effects of the O_3 were achieved with the shortest time and only slight reductions in aerobic bacteria, and yeasts, and molds counts were observed with a three-minute treatment. On the other hand, O_3-treated samples for three and five minutes presented the lowest counts of psychrotrophic bacteria immediately after washing and during 14 days of cold storage.

In contrast, the exposure of peppers to gaseous O_3 was effective in reducing both pathogenic and spoilage microbial populations, and aflatoxins (Inan et al., 2007; Akbas and Ozdemir, 2008b; Horvitz and Cantalejo, 2010b). Flaked red peppers inoculated with *E. coli* and *B. cereus* were exposed to gaseous O_3 for 360 minutes, achieving reductions in *E. coli* counts of less than 1, about 1.7 and 2 log units at O_3 concentrations of 0.1, 0.5, and 1 ppm, respectively. At the same time, only the highest concentration was effective against *B. cereus*, reducing the counts in 1.5 log units (Akbas and Ozdemir, 2008b).

Higher concentrations of O_3 were needed against *B. cereus* spores. In this case, the treatment for 360 minutes decreased the counts by about 1 and 1.5 log units with 5 and 7 ppm O_3, respectively. Lower concentrations of the gas had no positive

effects, while no further reductions were observed by increasing the O_3 level to 9 ppm.

In another set of experiments, minimally processed red bell peppers were treated with 0.7 ppm of O_3 for one, three, and five minutes before packaging (Horvitz and Cantalejo, 2010b). These authors reported a reduction of approximately 2.56 log units in the aerobic mesophilic counts of the treated samples after 14 days of cold storage and regardless of the duration of the treatment. On the other hand, the magnitude of inhibition of psychrotrophic bacteria increased with greater treatment duration with a maximum reduction of 3.3 log units when O_3 was applied for five minutes. Finally, neither yeasts nor moulds were observed in the ozonated samples immediately after the treatment, and the counts remained lower than in the control group all through the storage period. In contrast, when O_3 dose was increased to 1 ppm, all the microbial counts were lower in the control fruit, indicating an excessive concentration of the gas and injuries to the tissues of the treated samples, which in turn enhanced microbial growth (Horvitz, 2009).

This sanitizer was also studied for its effects in degrading aflatoxins. In both, flaked and chopped red peppers, ozone treatments (16, 33, and 66 mg/L, applied for 7.5, 15, 30, and 60 minutes at 25°C and 60% RH) showed positive results, degrading aflatoxin B_1 between 80 to 93% of its initial content, to a greater extent with increased O_3 concentration and time of treatment (Inan et al., 2007).

Related to the physicochemical parameters, neither aqueous (Horvitz and Cantalejo, 2010a) nor gaseous (Horvitz and Cantalejo, 2010b) O_3 affected the weight loss, pH, O_2, and CO_2 evolution of the treated samples. Conversely, washing minimally processed red peppers with ozonated water (1 ppm) enhanced softening, especially with the longer times of exposure. In general, O_3-treated peppers presented lower °hue values than the control ones, but no surface discoloration was detected and no significant variation in color quality was found (Inan et al., 2007; Horvitz and Cantalejo, 2010b). Sensory quality was not affected by O_3 concentrations up to 1 ppm, but flavor, appearance, and overall palatability punctuations were slightly lower than in the control samples when O_3 concentration was of 5 ppm or more (Akbas and Ozdemir, 2008b).

Tomatoes

According to Aguayo et al. (2006), an intermittent exposure to O_3 during storage could be used to reduce microbial growth and maintain the sensory quality of fresh-cut tomatoes. Effectively, these authors observed a reduction in both bacterial and fungal counts on sliced tomatoes subjected to 4 μL/L O_3 during 30 minutes each three hour, after 15 days of cold storage. Decreases of 1.07 and 1.27 log units were achieved for mesophilic and psychrotrophic bacteria, respectively, while, in contrast with the control group, no fungal development was detected in the O_3-treated slices.

Concerning the metabolic behavior and the physicochemical and sensorial quality of the tomatoes, the respiration rate was stimulated by the O_3 treatment the first two days of storage, but then decreased to lower levels than in the control fruits. Ethylene production was reduced and, firmness, juice color, TA and SSC of the slices were not affected by the exposure to the gas. On the other hand, control samples had a lower fructose, glucose, and ascorbic and fumaric acids content than ozonated ones, which was attributed to the lower metabolism of the treated samples. Finally, even when O_3-treated slices showed a loss of aroma, they maintained a better appearance and overall quality than control slices all through the storage period.

Watermelon

Fonseca and Rushing (2006) compared the effects of a non-aqueous treatment with UV-light with washing watermelon cubes with tap, ozonated (0.4 μL/L, 3 minutes) and chlorinated water on the physicochemical, microbiological, and sensory quality of the produce. From the results of the study, the authors concluded that O_3 was not effective in reducing the microbial populations. Furthermore, the overall quality was lower in those cubes receiving aqueous treatments compared to the UV-irradiated ones or the unwashed controls. The differences between aqueous and non-aqueous treatments were attributed to mechanical damages incurred during the centrifugation process to remove excess water due to the delicate texture of the watermelon.

An alternative that could be tested to improve O_3 efficacy could be the washing of the whole fruits before cutting or the treatment of the cubes with gaseous O_3, which showed positive results in minimally processed cantaloupes and peppers, respectively (Selma et al., 2008a; Horvitz and Cantalejo 2010b).

In Table 9, the different research works dealing with ozone effects on microbial, physicochemical, and sensorial quality of minimally processed fruit vegetables are summarized.

OTHER

Mushrooms

Yuk et al. (2007) inoculated sliced enoki mushrooms with *E. coli* O157:H7 and *L. monocytogenes* prior to immersion for half, one, three, and five minutes in 1, 3, and 5 ppm ozonated water at 22°C without agitation. Treatment with 3 ppm O_3 for five minutes reduced the number of *E. coli* O157:H7 by 0.94 log units compared to the untreated control samples, while no effects were observed on *L. monocytogenes*, even at the greatest concentration (5 ppm) and the longest time of exposure (5 minutes). The efficacy of this treatment was enhanced by combination with 1% citric acid, achieving reductions of 2.26 and 1.33 log units for *E. coli* and *L. monocytogenes* populations, respectively. This effect was attributed to the lower pH of the water, which can increase the stability of the aqueous O_3. However, none of the treatments showed residual antimicrobial effects during 10 days

Table 9 Ozone treatment studies on minimally processed fruit vegetables

Vegetable	O_3 Treatment	Parameters studied	References
Cantaloupes	**Gaseous** O_3: 10,000 ppm O_3—30 min, 11°C	Firmness, color, SSC Spoilage microorganisms and *E. coli* inhibition Sensory analysis	Selma et al., 2008a
Peppers	**Gaseous** O_3: 16.33 and 66 mg/L—7.5, 15, 30 and 60 min	Aflatoxin detoxification, Color	Inan et al., 2007
	Gaseous O_3: 0.1 to 9 ppm—up to 360 min	*E. coli*, *B. cereus* and *B. cereus* spores inactivation Sensory analysis	Akbas and Ozdemir 2008b
	Gaseous O_3: 0.7 ppm—1, 3 and 5 min	Microbial inhibition Color, pH, texture	Horvitz and Cantalejo 2010a, 2010b
	Aqueous O_3: 1 ppm—1, 3 and 5 min	O_2 and CO_2 evolution Weight loss	Ketteringham et al., 2006
	Aqueous O_3: 0.30 to 3.9 mg O_3/L—20 s to 30 min	Aerobic mesophiles growth	Ketteringham et al., 2006
Tomatoes	Exposure to 4 ± 0.5 μL/L O_3 **gas**: 30 min every 3 h	Bacterial and fungal counts Color, texture Respiration rate Ethylene emission TA, SSC, organic acids, sugars Vitamin C content Sensory evaluation Weight loss	Aguayo et al., 2006
Watermelon	**Aqueous** O_3: 0.4 μL/L—3 min	Microbiological, physicochemical and sensory analysis	Fonseca and Rushing, 2006

of storage at 15°C, the need of maintaining strict hygiene during the disinfection and processing steps being remarked.

In another research, Escriche et al. (2001) studied the effects of gaseous ozone on physicochemical properties of mushrooms (*Agaricus bisporus* type Gurelan 55). Results from this study showed that the external browning rate was enhanced after the exposure to O_3 (100 mg/h, applied for 15 and 30 minutes in a sealed container of 0.0385 m^3), while the opposite occurred with the internal browning rate. Furthermore, decreases in L^* values were higher with longer exposures to the gas. On the other hand, O_3 did not affect either texture, maturity rate or weight loss during a storage period of seven days.

Black Peppers

Zhao and Cranston (1995) found that an immersion of black peppercorns in water sparged with ozonated air for 10 minutes (6.7 mg/L O_3 at an air flow rate of 6 L/min) was an effective treatment to reduce the total aerobic and anaerobic bacteria and mesophilic aerobic spore formers counts by 3–4 log units. Those authors also treated ground black pepper with ozonated air (40 mg/min) for up to 6 hours and observed reductions of more than 3 log units in the populations of *Salmonella* spp and *E. coli* after 60 minutes, and of *Penicillium* spp after 40 minutes. In the case of *Aspergillus* spp, the counts were reduced by more than four log units after 10 minutes of treatment. In general, ozone was more effective with higher moisture contents in the samples and most of the reductions occurred in the first two hours of exposure to the gas.

In another research work, Emer et al. (2008) also reported reductions in *E. coli* population of about 7 log units after whole black peppers inoculated with the pathogen were treated with 0.1, 0.5, and 1.0 ppm of ozone for 360, 240 and 120 minutes, respectively. On the other hand, to obtain similar reductions in ground black peppers, it was necessary to apply 0.1 and 0.5 ppm O_3 for 360 minutes or 1 ppm of the gas for 240 minutes. As it happened with ground pistachios, the surface area in contact with the gas resulted in a critical factor influencing the efficacy of the sanitizing treatment.

Compared with the control and regardless of the time of exposure, the organoleptic properties remained unchanged in both whole and ground peppers treated with the lowest O_3 concentration (Emer et al., 2008). In contrast, with 0.5 and 1 ppm O_3, no changes were detected until 120 minutes of treatment, but odor, color, and overall acceptability were affected when the time of exposure was increased either to 240 or 360 minutes. On the other hand, O_3 did not affect the volatile oil constituents of whole peppercorns but, on ground peppers, the O_3 destroyed some constituents, produced new ones, and changed the relative concentrations of individual components with respect to the control samples (Zhao and Cranston, 1995).

Alfalfa Sprouts

Sharma et al. (2003) obtained similar results by immersing alfalfa sprouts inoculated with *E. coli* O157:H7 in sterile deionized water or water containing an initial O_3 concentration of 21 ppm, for 2 to 64 minutes. In this study, the exposure to O_3 yielded population reductions ranging from 0.67 to 0.85 log

units with similar reductions for all the contact times. These authors attributed this effect to the rapid decrease in ozone concentration upon contact with the alfalfa sprouts. The efficacy of O_3 was improved by continuously sparging the washing water with gaseous O_3. In these conditions, the reductions in populations of the pathogen increased from 0.83 to 2.2 log units for treatments of 2 and 64 minutes, respectively. At the same time, and except for the two-minute treatment, significantly lower reductions were observed for the control samples.

Moreover, ozonated water did not have detrimental effects on the visual quality of the sprouts. Effectively, O_3-treated samples appeared whiter and cleaner than the control ones and there was no breakage either of the cotyledons or of the hypocotyls in any treatment (Sharma et al., 2003).

CONCLUSIONS

Novel industrial applications and improvements in ozone technology together with new regulatory actions worldwide have emerged in recent years, making its use in the food industry easier. In this regard, the reviewed research works could be used as a basis for further studies on technological uses in the industry, integrating O_3 application on the food processing chain.

However, discrepancies regarding the efficacy of the O_3 are often found in the bibliography and further research is still needed. These differences could be attributed to a great variability in the conditions of the research work: the feeding gas and the method used for ozone generation and application, the O_3 concentration, and the exposure time to the gas as well as the way in which the produce is packed. Likewise, the environmental conditions such as temperature, relative humidity, and pH, and the commodity and microbial group studied are important factors to be considered.

In this sense, standardization in the working conditions and in the units to measure ozone concentration will be useful to better understand the mode of action and the effects of ozone not only on fruits and vegetables but also on other food products, such as fish, meat, and poultry among others. As parts per million (ppm) are the most frequently used units, we suggest the expression of the O_3 concentrations in these units. For this purpose, the conversion could be made by means of tables that are already published, such as those available at the following addresses:

http://www.lenntech.com/calculators/ppm/converter-parts-per-million.htm, http://www.drydenaqua.com/ozone/data/useful_ozone_conversion_factors.htm, http://www.ozonesolutions.com/Ozone_Conversions.html and http://www.ozonelab.com/downloads/index.htm, among others.

Consequently, it would be possible to improve the potential of ozone as a sanitizer in the food industry.

Lastly, before O_3 is going to be applied, it must be kept in mind that the exposure to high concentrations of this gas can cause deleterious effects on both, the workers and the treated produce. Thus, strict safety measures should be established to avoid negative effects from treatments with this sanitizer.

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