5 Ozone in Fruit and Vegetable Processing

B.K. Tiwari and K. Muthukumarappan

5.1 Introduction

Ozone finds wide application in the food industry, including surface decontamination of fruits and vegetables, drinking water disinfection and wastewater treatment (Guzel-Seydim et al. 2004; Karaca and Velioglu 2007). Ozone is applied in either gaseous or aqueous form. Minimising the occurrence of pathogenic and spoilage microorganisms in fruits, vegetables and their products is a primary food-safety concern. Consumer preference for minimally processed foods and foods free of chemical preservatives, as well as recent outbreaks of foodborne pathogens, identification of new food pathogens and the passage of new legislation such as the Food Quality Protection Act in the USA, have all stimulated demand for novel food processing and preservation systems. Several incidents of foodborne disease have been associated with fruit and vegetable products. In 1991, an outbreak of *Escherichia coli* O157:H7 infections and haemolytic uremic syndrome was linked to traditionally pressed apple cider. In the USA 21 juice-associated outbreaks were reported to the CDC (Centers for Disease Control and Prevention) between 1995 and 2005 (Vojdani et al. 2008). Recent outbreaks have shown that fruit juices can be vehicles for foodborne pathogens (CDC 1996 and 1999). *E. coli* O157:H7 is an enteric pathogen with a low infectious dose, which usually causes haemorrhagic colitis, but also has the potential to cause haemolytic uremic syndrome in young children and the immunocompromised (Boyce et al. 1995).

Ozone is a powerful broad-spectrum antimicrobial agent that is active against bacteria, fungi, viruses, protozoa, and bacterial and fungal spores (Khadre et al. 2001) pertinent to fruits and vegetables and their products. Efficacy against both Gram-positive and Gram-negative bacteria and fungi is reported, as well as potential virucidal effects (Restaino et al. 1995). Apart from the wide spectrum of microbial inactivation, ozone also has the potential to kill storage pests and degrade mycotoxins. One of the potential advantages of ozone is that excess ozone autodecomposes rapidly to
produce oxygen, and thus generally leaves no residue in food. However, ozone reactions with organic compounds can lead to new, partially oxidised compounds, some of which may remain in the food.

Its efficacy against a wide range of microorganisms, including bacteria, fungi, viruses, protozoa and bacterial fungal spores, has been reported (Restaino et al. 1995; Khadre et al. 2001; Cullen et al. 2009). Such advantages make ozone attractive to the food industry and consequently it has been affirmed as Generally Recognised as Safe (GRAS) for use in food processing (Graham 1997) and was approved as an antimicrobial food additive in 2001 (FDA 2001). This chapter outlines the efficacy of ozone for the storage and preservation of fruits, vegetables and their products, the effect of ozonation on product quality, and the current status of ozone application in fruit and vegetable processing.

5.2 Applications in fruit and vegetable processing

Ozone as a disinfecting agent has widespread application to assure safety and quality. It has several advantages over conventional disinfectant agents such as chlorine, chlorine dioxide, calcium hypochlorite, sodium chlorite, peroxyacetic acid and sodium hypochlorite. Some of these agents are inefficient against specific organisms. Table 5.1 shows the advantages and disadvantages of several disinfectants used in the fruit and vegetable processing industries. Ozone is preferred over most popular disinfectants, such as chlorine, because of the relatively low inactivation rate of chlorine at concentrations limited by regulation. The main purposes of ozone application at the postharvest stage of fruit and vegetable processing are: inactivation of pathogenic and spoilage microorganisms, and destruction of pesticide and chemical residues.

5.2.1 Surface decontamination

Traditionally, ozone treatment within the fruit and vegetable processing industry has been carried out for surface decontamination of whole fruits and vegetables by either gaseous treatment or washing with ozone-containing water. Table 5.2 shows some examples of the effects of aqueous and gaseous ozone treatment on fruit and vegetable preservation and quality.

Aqueous ozone

Ozone has been used routinely for washing and storage of fruits and vegetables (Liangji 1999; Karaca and Velioglu 2007). Water containing ozone has been applied to fresh-cut vegetables for sanitation purposes, reducing microbial populations and extending shelf life (Beltrán et al. 2005a,b). Treatment of apples with ozone resulted in lower weight loss and spoilage (Achen and Yousef 2001). Increased shelf life of apples and oranges following ozone treatment has been attributed to the oxidation of ethylene.
Table 5.1 Advantages and limitations of disinfection methods proposed for fresh-cut organic vegetables (Olmez and Kretzschmar 2009). (Reprinted from LWT – Food Science and Technology, Volume 42, Issue Number 3, Hülya Ölmez, Ursula Kretzschmar, Potential alternative disinfection methods for organic fresh-cut industry for minimising water consumption and environmental impact, 686–93, 2009, with permission from Elsevier.)

<table>
<thead>
<tr>
<th>Disinfectant agent</th>
<th>Advantages</th>
<th>Disadvantages/limitation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorine (hypochlorite)</td>
<td>Low cost, Easily available</td>
<td>Hazardous DBP at high levels, Reacts with organic matter, in some cases leads to the production of toxic compounds, Efficacy is affected by the presence of organic matter, Corrosive, Activity pH dependent, Not allowed for organic products</td>
</tr>
<tr>
<td>Ozone</td>
<td>High antimicrobial activity, Short contact time, GRAS substance, No residue problem, No hazardous DBP formation, No need to store hazardous substances, Lower running cost, Requires onsite generation</td>
<td>Toxic when inhaled, Requires monitoring in indoor applications, Corrosive above 4 ppm, Higher initial investment cost</td>
</tr>
<tr>
<td>Chlorine dioxide</td>
<td>Higher antimicrobial efficacy at neutral pH than chlorine, Effectiveness less pH dependent than that of chlorine, Fewer potentially hazardous DBP formation than chlorine, Less corrosive than chlorine and ozone, Requires onsite generation</td>
<td>Not efficient at permitted levels for fresh produce, Explosive, Only allowed in whole produce, Final water rinsing is required after treatment, More iodinated DBP formation than chlorine if iodide ion is present in water, Formation of specific byproducts, chlorite and chlorate, Requires monitoring in indoor applications, Not allowed for organic products</td>
</tr>
<tr>
<td>Organic acids</td>
<td>Easy to use, No toxicity, Allowed for organic products</td>
<td>Long contact time, not relevant to the industry, Interferes with the sensory quality, Relatively lower antimicrobial efficacy</td>
</tr>
<tr>
<td>Peroxyacetic acid</td>
<td>Efficacy is not affected by the organic load of water, Efficacy unaffected by temperature changes, No harmful DBP formation, Not corrosive at permitted levels (&lt;80 ppm)</td>
<td>Low antimicrobial efficacy at permitted levels for vegetables, Not allowed for organic products</td>
</tr>
</tbody>
</table>

(Continued)
Table 5.1  (continued)

<table>
<thead>
<tr>
<th>Disinfectant agent</th>
<th>Advantages</th>
<th>Disadvantages/limitation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrogen peroxide</td>
<td>No residue problem</td>
<td>Low antimicrobial efficacy</td>
</tr>
<tr>
<td></td>
<td>Easy to use</td>
<td>Long contact time</td>
</tr>
<tr>
<td></td>
<td>Low cost</td>
<td>Phytotoxic, negative impact on overall quality</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Requires the removal of residual H₂O₂ after processing</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Not allowed for organic products</td>
</tr>
</tbody>
</table>

Table 5.2  Effect of ozone on fruit and vegetable preservation and quality.

<table>
<thead>
<tr>
<th>Food product</th>
<th>Target microbial population</th>
<th>Quality attributes</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aqueous ozone treatment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lettuce</td>
<td>Shigella sonnei (1.8 LR)</td>
<td></td>
<td>Gil et al. (2006)</td>
</tr>
<tr>
<td>Iceberg lettuce</td>
<td>APC (1.1 LR)</td>
<td>Shelf life (↑), visual sensory quality (↑)</td>
<td>Garcia et al. (2003)</td>
</tr>
<tr>
<td>Apple</td>
<td>E. coli O157:H7 (3.7 LR)</td>
<td></td>
<td>Achen and Yousef (2001)</td>
</tr>
<tr>
<td></td>
<td>E. coli O157:H7 (2.6 LR)</td>
<td></td>
<td>Achen and Yousef (2001)</td>
</tr>
<tr>
<td>Coriander (Coriandrum sativum L.)</td>
<td>TPC (↓)</td>
<td>Aroma (-), flavour (-), overall quality (↑)</td>
<td>Feng et al. (2004)</td>
</tr>
<tr>
<td>Lettuce</td>
<td>E. coli O157:H7 (1.42 LR)</td>
<td>Colour (↓)</td>
<td>Singh et al. (2002)</td>
</tr>
<tr>
<td>Baby carrot</td>
<td>E. coli O157:H7 (1.8 LR)</td>
<td>Colour (↓), overall quality (↓)</td>
<td>Singh et al. (2002)</td>
</tr>
<tr>
<td>Watermelon</td>
<td>APC (1–1.5 LR)</td>
<td>Colour (↓), overall quality (↓)</td>
<td>Fonseca and Rushing (2006)</td>
</tr>
<tr>
<td>Celery</td>
<td>Total bacteria (1.15 LR)</td>
<td>Total sugar (-), colour (-)</td>
<td>Zhang et al. (2005)</td>
</tr>
<tr>
<td>Lettuce</td>
<td>PPO (↓)</td>
<td>Antioxidants (-), vitamin C (↓), visual appearance (-)</td>
<td>Beltran et al. (2005a)</td>
</tr>
<tr>
<td>Fresh-cut potato strips</td>
<td>LAB(↓), coliforms (↓) and anaerobic bacteria (↓)</td>
<td>Shelf life (↑), non-enzymatic browning (↑)</td>
<td>Beltran et al. (2005b)</td>
</tr>
<tr>
<td>Blueberries</td>
<td>E. coli O157:H7 (3.0 LR)</td>
<td>Colour (-)</td>
<td>Bialka and Demirci (2007)</td>
</tr>
</tbody>
</table>

Gaseous ozone treatment

| Lettuce | E. coli O157:H7 (1.84 LR) | Colour (↓) | Singh et al. (2002) |
| Baby carrot | E. coli O157:H7 (2.64 LR) | Colour (↓) | Singh et al. (2002) |
| Green pepper | E. coli O157:H7 (5 LR)   |            | Han et al. (2002) |
(Continued)
Fungal deterioration of blackberries and grapes was decreased by ozonation of the fruits (Beuchat 1992). Ozone-containing water was found to reduce bacterial content in shredded lettuce, blackberries, grapes, black pepper, broccoli, carrots and tomatoes (Kim et al. 1999b; Barth et al. 1995; Zhao and Cranston 1995; Sarig et al. 1996). Microbial studies typically show a 2 log reduction of total counts and significant reductions in spoilage and potentially pathogenic species most commonly associated with fruit and vegetable products.

Selma et al. (2007) reported that ozone treatments at levels of 1.6 and 2.2 ppm for 1 minute decreased *Shigella sonnei* population in water by 3.7 and 5.6 log colony forming unit (CFU)/mL, respectively. In addition, *S. sonnei* counts were reduced by 1.8 log units in lettuce treated with 5 ppm of ozone for 5 minutes (Selma et al. 2007). Oztekin et al. (2006) reported the effects of ozone treatment on the microflora of dried figs, where the application of gaseous ozone at 5 or 10 ppm for 3 to 5 hours resulted in significant reductions in total bacteria, coliform and yeast/mould counts. Najafi and Khodaparast (2009) concluded that a minimum of 1 hour of ozone treatment at 5 ppm could be successfully used to reduce both coliform and *Staphylococcus aureus* populations on date fruits, but that longer exposure times were required for elimination of the total mesophilic bacteria as well as yeast/mould present.

Washing of fruits and vegetables was also reported to degrade pesticide residues. Wu et al. (2007) reported that rinsing at a dissolved ozone concentration of 1.4 mg/L for 15 minutes effectively removes 27–34% of residual pesticide from vegetables. However, higher degradation of pesticides residues can be obtained with an increase in ozone concentration (Ou-Yang et al. 2004; Ong et al. 1996). Inan et al. (2007) investigated the

<table>
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<th>Table 5.2 (continued)</th>
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<tbody>
<tr>
<td><strong>Food product</strong></td>
</tr>
<tr>
<td>Pistachio (<em>Pistachio vera</em> L.)</td>
</tr>
<tr>
<td>Fig</td>
</tr>
<tr>
<td>Blueberries</td>
</tr>
</tbody>
</table>

FFA, free fatty acid; APC, aerobic plate count; AA, ascorbic acid; PPO, polyphenol oxidase; LR, log reduction. (x) significant difference; (↑) increases; (↓) decreases; (−) no change.
efficacy of ozone for the degradation of aflatoxin B1 in flaked and chopped red peppers. They observed 80 and 93% degradation of aflatoxin B1 at an ozone concentration of 33 and 66 mg/L for 60 minutes, respectively.

Wang et al. (2004) employed tap water, acidic electrolysed water (AEW), aqueous ozone, chlorinated water and aqueous ozone followed by AEW (sequential wash) for treatment of fresh-cut coriander samples. They observed that the sequential wash – that is, aqueous ozone followed by AEW – is more effective in initial microbial count reduction and maintains low microbial growth during storage at 0 °C for 14 days. However, the combination of ozone and AEW led to more tissue injury, which influences the overall quality of coriander, whereas ozone treatment alone achieved the highest overall quality of coriander during storage and maintained the typical coriander aroma.

Figure 5.1 shows an effective ozone washing system, which includes a shower as a prewashing step to remove dirt and cell exudates from the cut surfaces. This step is followed by the immersion of the product in a washing tank, which contains ozone as the sanitising agent (Gil et al. 2009). A rinse step is optional depending on the sanitising agent. It is recommended that water flows in the opposite direction to the movement of produce through the different unit operations. Thus, water in the sanitising tank could be recirculated for use in the prewashing step (Figure 5.1). The same applies to the rinse water, which could be incorporated into the sanitising tank after the shower in a continuous process. Water disinfection remains an essential activity in the fresh-cut industry and is possible with an efficient disinfection strategy such as chlorine, ozone and AOPs in a recirculated system (Figure 5.1) (Gil et al. 2009).
Gaseous ozone

The use of gaseous ozone to reduce *Bacillus* spp. and *Micrococcus* counts in peas and beans by up to 3 log units, depending on ozone concentration, temperature and relative humidity (RH) conditions, was reported by Naitoh et al. (1988). Fan et al. (2007) reported that gaseous ozone effectively inactivated *Listeria innocua* on solid media at concentrations of 50 and 100 nL/L during short exposure times at both 5 and 20 °C. The red colour of intact, whole berry fruit was optimum in 0.3 ppm ozone-treated samples during storage (Barth et al. 1995). It was also reported that the undesirable colour change from green to yellow in broccoli was significantly less pronounced for ozone-treated samples. However, ozone was reported to change the surface colour of products such as peaches (Badiani et al. 1995) and carrots (Liew and Prange 1994). The majority of gaseous ozone treatments are reported to be during storage, as discussed in section 5.2.2.

5.2.2 Storage in ozone-rich atmospheres

Apart from surface decontamination of fruits and vegetables, storage of fruits and vegetables in ozone-rich atmospheres has been reported to reduce or eliminate odour, and to control spoilage caused by microbial and fungal pathogens. Continuous exposure of fresh commodities to ozone during storage is reported to reduce postharvest decay and to reduce microbial spoilage of fruits and vegetables (Liew and Prange 1994; Barth et al. 1995; Sarig et al. 1996; Perez et al. 1999; Palou et al. 2002; Aguayo et al. 2006; Tzortzakis et al. 2007a,b). Applications of ozone-rich atmospheres have been studied for apples, cherries, carrots, kiwi, onions, peach, plum, potatoes, table grapes, tomatoes, blackberries and strawberries (Table 5.3).

Ozone can be used as a relatively brief prestorage treatment in air or water, or it can be added continuously or intermittently to the storage room atmosphere throughout the storage period to prevent or delay fruit decay (Skog and Chu 2001; Palou et al. 2003; Cayuela et al. 2009). Hildebrand et al. (2008) observed a reduction in postharvest decay when carrots inoculated with *S. sclerotiorum* and *B. cinerea* were held in 115–530 nL/L ozone at 10 °C for 20 days.

Reported studies show that the effect of ozone during storage is variable and strongly depends on the type of microorganism, commodities and storage conditions. For example, Forney et al. (2003) observed a decay resistance towards *B. cinerea* in carrots treated with 1000 nL/L ozone for 2 or 4 days, however they did not observe a decay resistance towards *S. sclerotiorum*. Similarly, Skog and Chu (2001) reported that an ozone concentration of 0.04 μL/L has the potential to extend the storage life of broccoli and cucumbers stored at 3 °C. However, they did not observe similar effects for mushrooms stored at 4 °C or cucumbers stored at 10 °C. Ogawa et al. (1990) reported the inactivation of *B. cinerea* spores in tomato fruits after
Table 5.3 Application of ozone during storage of fruits and vegetables.

<table>
<thead>
<tr>
<th>Food product</th>
<th>Storage conditions</th>
<th>Target Microbial population</th>
<th>Salient findings</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carrot</td>
<td>Ozone concentration of 0 (control), 75, 15, 30 or 60 μL/L. Treatment chambers were flushed with a total flow rate of 0.5 L/min (air and ozone) for 8 hours daily for 28 days. The experiment was repeated twice at storage temperatures of 2, 8 and 16 °C.</td>
<td>Botrytis cinerea Pers. and Sclerotinia sclerotiorum de Bary</td>
<td>A 50% reduction of daily growth rates of both fungi at the highest ozone concentration indicated that ozone was fungistatic. Carrot respiration rate, electrolyte leakage and total colour differences increased with ozone concentration. Ozone-treated carrots were lighter (higher L* values) and less intense (lower chroma values) in colour than control carrots.</td>
<td>Liew and Prange (1994)</td>
</tr>
<tr>
<td>Carrot</td>
<td>Ozone concentration of 50 ± 10 nl/L ozone during storage for up to 6 months at 0.5 °C and &gt;95% relative humidity.</td>
<td>Sclerotinia sclerotiorum and Botrytis cinerea</td>
<td>Reduced lesion size and aerial mycelium of both pathogens. Ozone-induced injury, appearing as blotches of brownish discoloured periderm. Ozone treatment had no effect on fresh weight loss, sprouting of carrot crowns or concentrations of glucose, fructose, sucrose or galactose.</td>
<td>Hildebrand et al. (2008)</td>
</tr>
<tr>
<td>Blackberry</td>
<td>Stored for 12 days at 2 °C in 0.0, 0.1 and 0.3 ppm ozone.</td>
<td>Fungal decay (Botrytis cinerea)</td>
<td>Ozone storage suppressed fungal development for 12 days. Ozone storage did not cause observable injury or defects. On day 12, anthocyanin content of juice was similar to initial levels for all treatments. Surface colour was better retained in 0.1 and 0.3 ppm-stored berries by 5 days and in 0.3 ppm berries by 12 days, by hue angle values. POD was greater in controls and 0.1 ppm samples, and was lowest in 0.3 ppm fruits by 12 days.</td>
<td>Barth et al. (1995)</td>
</tr>
<tr>
<td>Strawberry (Fragaria × ananassa Duch. cv. Camarosa)</td>
<td>Strawberry fruits were stored at 2 °C in an atmosphere containing ozone (0.35 ppm). After 3 days at 2 °C, fruits were moved to 20 °C to mimic retail conditions.</td>
<td>Fungal decay (B. cinerea)</td>
<td>Ozone treatment was ineffective in preventing fungal decay in strawberries after 4 days at 20 °C. Significant differences in sugars and ascorbic acid (reduced by 3 times) content were found in ozone-treated strawberries.</td>
<td>Perez et al. (1999)</td>
</tr>
</tbody>
</table>
A detrimental effect of ozone treatment on strawberry aroma was observed, with a 40% reduced emission of volatile esters in ozonated fruits.

Grape and Peach
Continuous ozone exposure at 0.3 ppm (v/v) for 4 weeks at 5°C and 90% RH.

Monilinia fructicola, Botrytis cinerea, Mucor piriformis or Penicillium expansum

Ozone exposure did not significantly reduce the incidence and severity of decay caused by these fungi, with the exception of brown rot.

Continuous ozone exposure at 0.3 ppm increased water loss after 5 weeks of storage.

No phytotoxic injuries of fruit tissues were observed in ozonated or ambient atmosphere treatments.

Palou et al. (2002)

Whole and fresh-cut Tomato
Humidified flow of ozone-enriched air applied cyclically (4 ± 0.5 μL of O₃ for 30 minutes every 3 hours) stored up to 15 days at 5°C

Mesophilic and psychrotrophic aerobic bacteria, yeasts and moulds count

Significant reduction in bacteria (1.1–1.2log₁₀ units) and fungi (0.5log₁₀ units).

In whole tomatoes, O₃ maintained the tissue firmer than in control fruit, while no influence was found on slices.

No significant changes in appearance and overall quality in slices but a slight reduction in aroma.

No significant damage or off-flavour in slices or whole tomatoes.

Aguayo et al. (2006)

Tomato, Strawberry, Table Grape and Plum
Chilled storage (13 °C) and exposed to 'clean air' or low-level ozone enrichment (0.1 μmol/mol).

Botrytis cinerea (grey mould)

Ozone-enrichment resulted in a substantial decline in spore production as well as visible lesion development.

Tzortzakis et al. (2007)

Tomato
Ozone concentrations between 0.005 (control) and 5.0 μmol/mol up to 13 days at 13°C.

Alternaria alternata or Colletotrichum coccodes

Significant reduction in fungal lesion development.

Concentration-specific impacts on fungal lesion development.

Tzortzakis et al. (2008)

RH, relative humidity; POD, peroxidases; L*, lightness.
ozone treatment, whereas Liew and Prange (1994) concluded that the effect of ozone on *B. cinerea* was fungistatic but not fungicidal in treated carrots. Sharpe et al. (2009) investigated the effect of gaseous ozone on spore viability of *B. cinerea* and mycelial growth of *B. cinerea* and *S. sclerotiorum* for apples, grapes, highbush blueberries and carrots. They observed a significant reduction in spore viability of *B. cinerea* of over 99.5% and a reduction in the aerial mycelium from 4.7 mm in the control to less than 1 mm after exposure to 450 or 600 ppb ozone for 48 hours at 20 °C (Figure 5.2).

Furthermore, high ozone concentrations during storage may cause surface discolouration (blotches). Studies have shown reductions in decay of blackberries during continuous ozone treatment (Barth et al. 1995) and in mould inhibition and decay of onions. A delay of the growth of green and blue mould was observed in ozonated citrus fruit (Palou et al. 2001).
Apart from reduction in microbial decay, ozone is reported to be an effective agent in removing ethylene from the atmosphere during apple and pear storage without a significant change in quality attributes (Skog and Chu 2001). Exposure of horticulture crops to ozone can reduce postharvest decay and may be effective in reducing application of field-applied fungicides used to control these pathogens.

### 5.2.3 Ozone in fruit and vegetable juice processing

Currently the practical application of ozonation to fruit juices is still in its infancy. Ozonation of liquid phases is most frequently accomplished by injecting ozone gas (mixtures of air/ozone or oxygen/ozone) through a sparger into a liquid. Usually the studies on ozone absorption in the aqueous systems are carried out in stirred-tank reactors or bubble columns (Cullen et al. 2009). The approval of ozone as a direct food additive (FDA 2001) led to the application of ozone for processing of various fruit juices, including: apple cider (Steenstrup and Floros 2004; Choi and Nielsen 2005) orange juice (Angelino et al. 2003; Tiwari et al. 2008; Patil et al. 2009a,b), blackberry juice (Tiwari et al. 2009a) and strawberry juice (Tiwari et al. 2009b). Ozonation of fruit juices is reported to meet the FDA’s requirement of a mandatory 5 log reduction of the most resistant pathogens (E. coli, Salmonella, Listeria monocytogenes).

Apart from microbial inactivation, ozone treatment of apple juice has also been reported for destruction of mycotoxins (Cataldo 2008). Mycotoxins have been found to occur in a number of foods, including apple juice. Patulin is a predominant mycotoxin in apple juice with an FDA action level of 50 μg/L. Patulin has carcinogenic properties and it survives conventional pasteurisation processes. Cataldo (2008) reported that a moderate ozone treatment of apple juice may become a standard industrial practice to reduce or eliminate the patulin toxin from juices. Similarly, Ashirifie-Gogofio et al. (2009) reported that an apple juice model system (0.5% malic acid, buffered at pH 3.5–4.0) with an initial level of 1000 ppb patulin was degraded to <50 ppb in 20, 15 and 10 minutes for 350 ppm, 1500 ppm and 2500 ppm O₃, respectively. The efficiency of ozone in mycotoxin degradation is due to the presence of a polyketide lactone, which makes it highly vulnerable to oxidation. Hence ozone treatment effectively degrades mycotoxins.

### 5.3 Efficacy of ozone

Efficacy of ozone is affected by both extrinsic and intrinsic factors (Table 5.4) and it is difficult to predict ozone behaviour in the presence of specific compounds, other ingredients and environmental factors such as medium pH, temperature and humidity. Residual ozone is the concentration of
Table 5.4 Factors influencing efficacy of ozone.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Factors</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Extrinsic factors</strong></td>
<td></td>
</tr>
<tr>
<td>Water quality</td>
<td>pH, temperature, turbidity, organic matter, oxidizable inorganic materials (e.g. ferrous iron, manganous, sulfide, etc.)</td>
</tr>
<tr>
<td>Ozone Concentration</td>
<td>Concentration, contact time</td>
</tr>
<tr>
<td>Decontamination</td>
<td>Application method (dipping, spraying and agitated, rubbed or static condition during exposure), produce/water ratio, single or multiple batches, rinse after sanitation, multiple washings</td>
</tr>
<tr>
<td>treatment</td>
<td></td>
</tr>
<tr>
<td><strong>Intrinsic factors</strong></td>
<td></td>
</tr>
<tr>
<td>Microbial load</td>
<td>Characteristics of microbial strain, physiological states of the bacterial cells, natural or inoculated microorganisms, population size</td>
</tr>
<tr>
<td>Food product</td>
<td>Type of fruit and vegetable, characteristics of the product surfaces (cracks, crevices, hydrophobic tendency and texture), relation weight and surface area</td>
</tr>
</tbody>
</table>

Ozone that can be detected in the medium after application to the target surface. Both the instability of ozone under certain conditions and the presence of ozone-consum ing materials affect the level of residual ozone available in the medium. Therefore, it is important to distinguish between the concentration of applied ozone and residual ozone necessary for effective disinfection. It is advisable to monitor ozone availability during treatment (Pascual et al. 2007).

Food components are reported to interfere with the bactericidal properties of ozone (Guzel-Seydim et al. 2004). Efficacy of ozone is demonstrated more readily when targeted microorganisms are suspended and treated in pure water or simple buffers (with low ozone demand) than in complex food systems, in which it is difficult to predict how ozone reacts in the presence of organic matter (Cho et al. 2003). Organic substances with high ozone demand in a medium may compete with microorganisms for ozone (Khadre et al. 2001). Hence, the presence of organic matter or dissolved solids in water intended for washing of fruits and vegetables may increase ozone demand and may form undesirable byproducts due to reaction with ozone. The formation of these byproducts may shorten the shelf life, change the organoleptic quality or jeopardise the safety of the final product (Khadre et al. 2001).

The effectiveness of ozone against microorganisms depends not only on the amount used, but also on the residual ozone in the medium and various environmental factors such as medium pH, temperature, humidity, additives (surfactants, sugars, etc.) and the amount of organic matter surrounding the cells (Restaino et al. 1995). For example, whole-fruit bubbling of ozone in stored apples inoculated with *E. coli* O157:H7 was found to be more effective than dipping apples in ozone-containing water.
Bubbling and dipping resulted in 3.7 log and 2.6 log reductions in counts of *E. coli*, respectively (Achen and Yousef 2001). A 1.3–3.8 log reduction range for *E. coli* at ozone concentrations of 0.3–1.0 ppm (O3 demand-free water) and pH of 5.9 was reported. Populations of *Leuconostoc mesenteroides* at similar treatment conditions were reduced by 1.3 to −7 log CFU/mL and ozone concentrations of 0.2–1.8 ppm yielded 0.7 to −7 log reductions in *L. monocytogenes* (Kim et al. 1999a). The effect of pH on ozone inactivation is mainly attributed to the fact that the ozone decomposition rate changes substantially with changes in pH. At high pH, the chain reactions of ozone decomposition result in the formation of numerous radical species with high oxidative capabilities.

\[
O_3 + OH^- \rightarrow HO_2^- + O_2 \\
O_3 + HO_2^- \rightarrow 'OH + O_2 + O_2
\]

Patil et al. (2010) studied the effect of pH (at levels of 3.0, 3.5, 4.0, 4.5 and 5.0) on the microbial safety of apple juice. Apple juice inoculated with *E. coli* strains (10^6 CFU/mL) was treated with ozone at a flow rate of 0.12 L/min and ozone concentration of 0.048 mg/min/mL for different time periods (0–18 minutes). The results revealed that pH had a significant effect on the ozone inactivation kinetics. The ozone treatment duration for achieving a 5 log reduction was faster (4 minutes) at the lowest pH (3.0) than at the highest pH (5.0) (18 minutes) studied. The higher inactivation rates observed at lower pH may be due to the synergistic effect of pH on inactivation kinetics.

Factors influencing the solubility, stability and reactivity of ozone may also affect the efficacy of ozone (Table 5.4). There is no consensus on the effect of temperature on the biocidal efficacy of ozone. For example, a reduction in the temperature of an aqueous medium increases ozone solubility and stability, augmenting its availability in the medium, and consequently efficacy rises. The simultaneous contribution of these two factors (solubility/stability and reactivity) to ozone efficacy can vary with experimental conditions, making it difficult to predict the influence of temperature on a particular application (Pascual et al. 2007).

It has been widely reported since the 1930s that high RH is required for microorganisms to be inactivated by ozone gas. The optimum RH level is 90–95%. However, in general ozone loses its bactericidal efficiency below 50% RH. The strong effectiveness of ozone gas at high RH levels is beneficial for sanitation of fruits and vegetables where environmental RH levels generally are over 80% (Han et al. 2002). At high RH, ozone gas is more effective as an antimicrobial agent than gaseous disinfectants, such as ethylene oxide and propylene oxide (Wiley 1994), but may be less effective than ClO₂ gas (Han et al. 2002). Decreasing pH is reported to increase ozone efficiency (Kuscu and Pazir 2004) because the concentrations of molecular ozone molecules, responsible for providing the ‘Ct value’ (mg/min/L), are
more stable at lower pH than at the higher pH ranges. Zhao and Cranston (1995) found that ozone gas treatment of black pepper at higher moisture content led to increased reductions in the microbial load.

The effectiveness of sanitisers can be affected by product surface features (Han et al. 2002) and ozone application methods, such as bubbling and agitation (Kim et al. 1999a). Variations in these factors will influence the antimicrobial activity of ozone. The degree of attachment of the microorganism to the food significantly influences the bactericidal effects of ozone. Microorganisms embedded in product surfaces are more resistant to ozone than those readily exposed. Application of aqueous ozone to products having smooth intact surfaces with low ozone demand (for example, fruits and vegetables) produced promising results (Kim et al. 1999b; Achen and Yousef 2001). Similarly, Kim et al. (1999b) reported that inactivation of microflora on food by ozone depends greatly on the nature and composition of the food surface, the type and load of microbial contaminant and the degree of attachment or association of microorganisms with the food. However, application must ensure direct contact of ozone with the target microbial cells. A variety of methods have been employed to accomplish this, including stirring, pumping, fluming, bubbling, sonication, abrasion and pressure washing (Kim et al. 2001).

5.4 Synergistic effects with ozone

The efficacy of ozonation may be increased by use in combination with other technologies. The disaggregating effect of ultrasound upon solid matter and on gas bubbles may improve efficacy by increasing surface area. Furthermore, ultrasound accelerates the sedimentation of oxidisable organic matter, thus reducing ozone demand. Williams et al. (2005) reported that combinations of hydrogen peroxide and ozone treatment followed by refrigerated storage caused greater than 5 log CFU/mL reduction of E. coli O157:H7 and Salmonella in apple cider and orange juice. Some microorganisms are sensitive to lower concentrations of oxidising agents when exposed to ultrasound, and the combined action of ultraviolet (UV) radiation with high-frequency ultrasound increases the rate of bacterial inactivation (Sierra and Boucher 1971). Employing such hybrid techniques can also reduce the dosage of the chemical disinfectant required. Thus by using the combination of ultrasonic sonotrode and ozone or hydrodynamic cavitation and ozone, the concentration of ozone required for disinfection may be significantly reduced to half or one-third, depending upon the type of microorganism.

The combination of hydrodynamic cavitation and ozone has proved to be an efficient method of water disinfection (Jyoti and Pandit 2004). Yuk et al. (2007) found that a combined ozone and organic acid treatment was more effective than individual application for control of E. coli O157:H7 on
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enoki mushrooms. Garcia et al. (2003) reported an improvement in microbial reduction and an extension of shelf life as determined by appearance of a ready-to-eat (RTE) salad lettuce by using a sequential combination of ozone and chlorine. They also observed a significant reduction in process water turbidity compared to chlorine treatment alone, which would facilitate increased water reuse (Strickland et al. 2010).

Ozone in combination with citric or oxalic acid as a fumigant is reported to reduce postharvest decay and pericarp browning of longan fruit. Whangchai et al. (2006) observed that longan fruit treated with ozone in combination with oxalic or citric acid reduces enzymatic browning and could be a partial alternative to sulfur dioxide fumigation for control of postharvest decay and browning. Pericarp browning has been attributed to oxidation of phenolics by polyphenol oxidase (PPO), producing brown-coloured byproducts (Ferrar and Walker 1996).

5.5 Effect of ozone on product quality and nutrition

Microbial studies to date typically show that the mandatory 5 log reductions of spoilage and potentially pathogenic species most commonly associated with fruit and vegetable juices may be achieved. A number of studies report the effects of ozone on quality parameters of treated fruits and vegetables (Zhang et al. 2005; Fonseca and Rushing 2006). The effects of ozone treatment on quality and physiology of various foods are reported in Table 5.2. Applying ozone at doses that are large enough for effective decontamination may change the sensory qualities of food. Ozone is not universally beneficial and in some cases may promote oxidative spoilage in foods. Surface oxidation, discolouration or development of undesirable odours may occur in substrates from excessive use of ozone (Khadre et al. 2001). Dock (1999) reported no detrimental change in the quality attributes of apple cider when it was treated with ozone.

5.5.1 Chemical attributes

No change in onion chemical composition and sensory quality was reported by Song et al. (2000). Ozone-containing water treatment resulted in no significant difference in total sugar content of celery and strawberries (Zhang et al. 2005) during storage. Ozonation is expected to lead to the loss of antioxidant constituents, because of its strong oxidising activity. However, ozone washing treatment was reported to have no effect on the final phenolic content of fresh-cut iceberg lettuce (Beltrán et al. 2005a). Contradictory reports were found in the literature regarding ascorbic acid. Decomposition of ascorbic acid in broccoli florets was reported after ozone treatment by Lewis et al. (1996), but Zhang et al. (2005) reported no significant difference in ascorbic acid content between treated and nontreated
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celery samples. Moreover, increases in ascorbic acid levels in spinach (Luwe et al. 1993), pumpkin leaves (Ranieri et al. 1996) and strawberries (Perez et al. 1999) were reported in response to ozone exposure. Slight decreases in vitamin C content were reported in lettuce (Beltrán et al. 2005a). Ozone treatments were reported to have minor effects on the anthocyanin contents of strawberries (Perez et al. 1999) and blackberries (Barth et al. 1995).

Beltrán et al. (2005a) compared the effect of ozone-containing water (10 mg/L/min, 20 mg/L/min, 10 mg/L/min ozone converted into a stronger oxidising moiety or moieties by UVC radiation, and chlorine 80 mg/L) and reported that ozone concentration of 10 ppm in water activated by UVC radiation aids in extending the shelf life of fresh-cut lettuce. They concluded that ozone-containing water activated with UVC radiation could be an alternative to chlorine for washing of shredded lettuce, by reducing microbial populations on the product but also by maintaining the visual quality and controlling browning without any detrimental effect on the antioxidant constituents when combining with active modified atmosphere packaging (MAP).

Recently researchers in Spain evaluated the effects of continuous and intermittent applications of ozone gas treatments, applied during cold storage to maintain postharvest quality during subsequent shelf life, on the bioactive phenolic composition of Autumn Seedless table grapes after long-term storage and simulated retail display conditions (Artes-Hernandez et al. 2007). They found that the sensory quality was preserved with both ozone treatments. Although ozone treatment did not completely inhibit fungal development, its application increased the total flavan-3-ol content at all sampling times. Continuous 0.1 μL/L O3 application also preserved the total amount of hydroxycinnamates, while both treatments assayed maintained the flavonol content sampled at harvest. Total phenolics increased after the retail period in ozone-treated berries. Therefore, the improved techniques tested to enhance the quality of Autumn Seedless table grapes during long-term storage seem to maintain or even enhance the antioxidant compound content.

5.5.2 Visual quality

Gabler et al. (2010) investigated the efficacy of ozone in controlling postharvest decay of table grapes and for the potential replacement of sulfur dioxide, which is used as a commercial fumigant. They observed that ozone fumigation with up to 10000 μL/L for up to 2 hours helps to control postharvest grey mould of table grapes caused by Botrytis cinerea (Figure 5.3). However, grapes stored in ozone-rich atmospheres may develop thin longitudinal darkened lesions (Figure 5.4). This injury is reported to be irregular and was not always associated with an ozone dose or cultivar (Gabler et al. 2010). Similarly, Tzortzakis et al. (2007b) observed a substantial decline in spore production and development of
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Figure 5.3  Influence of postharvest ozone fumigation on the natural incidence of postharvest grey mould among several table grape cultivars. Freshly harvested, organically grown table grapes were fumigated with 5000 μL L⁻¹ ozone for 60 minutes in a commercial ozone chamber and stored for 6 weeks at 0.5 °C. Each value is the mean of four replicates per treatment and each replicate box contained nine cluster bags with 1000 g of grapes each. For each cultivar, columns with different letters differ significantly at P ≤ 0.05 (Gabler et al. 2010). (Reprinted from Postharvest Biology and Technology, Volume 55, Issue 2, Franka Mlikota Gabler, Joseph L. Smilanick, Monir F. Mansour and Hakan Karaca, 85–90, 2010, with permission from Elsevier.)

Figure 5.4  Occasional injuries to Thompson Seedless grape cluster rachis after grapes were fumigated once with 5000 μL/L ozone for 1 hour. Grapes were stored for 7 days at 15 °C (Gabler et al. 2010). (Reprinted from Postharvest Biology and Technology, Volume 55, Issue 2, Franka Mlikota Gabler, Joseph L. Smilanick, Monir F. Mansour and Hakan Karaca, 85–90, 2010, with permission from Elsevier.)

visible lesions mainly due to B. cinerea (grey mould) in tomatoes, strawberries, table grapes and plums during chilled storage (13 °C) with low-level ozone-enrichment (0.1 μmol/mol). Martínez-Sánc et al. (2006) investigated the effect of several sanitisers (Figure 5.5) on the visual quality and colour of rocket leaves during storage in air and low O₂ (1–3 kPa) + high CO₂ (11–13 kPa) for 15 days at 4 °C. They observed that ozone effects were comparable with other sanitisers, except for Purac® (lactic acid) treated samples.
Texture or firmness is an important rheological property pertinent to fresh fruits and vegetables. Fruits and vegetables with a firm, crunchy texture are highly desirable because consumers associate these textural attributes with freshness and wholesomeness. The appearance of a soft or limp product may give rise to consumer rejection prior to consumption (Rico et al. 2007). Textural changes in fruits and vegetables could be due to various enzymatic and non-enzymatic processes. Ozone treatment of fresh fruit and vegetables either by washing or in storage consisting of ozone gas is reported to have significant effects on texture. Firmness of fresh coriander leaves was reported to decrease through washing with ozone-containing water compared to control. A decrease in firmness was also reported by washing

Figure 5.5  Effect of chlorine (sodium hypochlorite), 100 mg/L), ozone (10 mg/L), Purac (lactic acid, 20 mL/L), Sanova (acidified sodium chlorite, 250 mg/L) and Tsunami (peroxyacetic acid, 300 mL/L) sanitisers on the visual quality and colour of rocket leaves stored under air and low O₂ and high CO₂ (MAP). Values are the mean of three replicates (Martínez-Sánchez et al. 2006). (Reprinted from Postharvest Biology and Technology, Volume 42, Issue 1, Ascensión Martínez-Sánchez, Ana Allende, Richard N. Bennett, Federico Ferreres and María Isabel Gil, Microbial, nutritional and sensory quality of rocket leaves as affected by different sanitisers, 86–97, with permission from Elsevier.)

5.5.3 Texture

 Texture or firmness is an important rheological property pertinent to fresh fruits and vegetables. Fruits and vegetables with a firm, crunchy texture are highly desirable because consumers associate these textural attributes with freshness and wholesomeness. The appearance of a soft or limp product may give rise to consumer rejection prior to consumption (Rico et al. 2007). Textural changes in fruits and vegetables could be due to various enzymatic and non-enzymatic processes. Ozone treatment of fresh fruit and vegetables either by washing or in storage consisting of ozone gas is reported to have significant effects on texture. Firmness of fresh coriander leaves was reported to decrease through washing with ozone-containing water compared to control. A decrease in firmness was also reported by washing.
with chlorinated water (Wang et al. 2004). Another study conducted by Selma et al. (2008) reported nonsignificant changes in firmness of fresh-cut cantaloupe irrespective of gaseous ozone concentration (5000 or 2000 ppm) for 30 minutes during storage compared to control.

Change in texture during ozonation and subsequent storage may possibly be due to postharvest changes in cellulose and hemicellulose contents due to ozone application during MAP. This could be due to polymerisation and epimerisation of cellulose and hemicelluloses contents of cell walls inducing thickening of the cell walls, causing textural changes in fresh-cut green asparagus during storage after ozone treatment (An et al. 2007). An et al. (2007) reported an increase in cellulose, hemicelluloses and lignin content during MAP storage after pretreatment with aqueous ozone.

Ozonation of fruits has been reported to enhance firmness of citrus fruits and cucumbers compared to controls (Skog and Chu 2001). Ozone is reported to delay softening in strawberries during cold-room storage and storage at room temperature (Nadas et al. 2003). Wang et al. (2004) compared five washing treatment systems including tap water, AEW, ozone-containing water, chlorinated water and aqueous ozone followed by AEW (sequential wash) on the firmness of coriander packaged in polyethylene bags and stored at 0 °C for 14 days. They observed a slight decrease in firmness of treated samples compared to the control on day 0, with no significant differences in firmness among chlorine, ozone, AEW and the sequential treatments at day 0. They observed a gradual decrease in firmness during storage, which may be attributed to the tissue injury caused by the treatments.

Ozone is a strong oxidising agent which can cause oxidation of feruloylated crosslinkages or phenolic crosslinkages among cell-wall pectin, structural proteins or other polymers, and thereby change the firmness of the product (Heun Hong and Gross 1998). Similarly, Forney et al. (2003) observed a reduction in firmness of ozone-treated carrot firmness during 8 weeks of storage. Aguayo et al. (2006) observed no significant changes in the firmness of tomato slices during treatment, whereas they observed a reduction of about 9.2, 16 and 35% in firmness after 5, 12 and 15 days of storage at 5 °C.

5.5.4 Sensory quality

The most notable effect of ozone on the sensory quality of fruits reported in the literature is the loss of aroma. Ozone-enriched cold storage of strawberries resulted in reversible losses of fruit aroma (Nadas et al. 2003; Perez et al. 1999). This behaviour is probably due to the oxidation of volatile compounds. However, Tzortzakis et al. (2007a) did not observe any significant changes in tomato fruit weight, antioxidant status, CO₂/H₂O exchange, ethylene production or in organic acid, vitamin C (pulp and
seed) or total phenolic content when exposed to ozone concentrations ranging between 0.005 and 1.0 μmol/mol at 13°C and 95% RH. Similar results were reported by Kute et al. (1995) for strawberry exposed to ozone concentrations between 0.3 and 0.7 μmol/mol for up to 1 week. Applying ozone at doses that are large enough for effective decontamination may change the sensory qualities of these products.

5.6 Conclusion

The effectiveness of ozone against microorganisms present in food systems depends on several factors, including the amount of ozone applied, the residual ozone in the medium, various environmental factors such as medium pH, temperature, humidity and additives (surfactants, sugars, etc.), and the amount of organic matter surrounding the cells (Pascual et al. 2007). To facilitate enhanced control of both quality and safety parameters of ozone-treated foods, mathematical models incorporating various independent factors governing ozone processing are required to describe biochemical reactions and microbial inactivation. Based on these modelling approaches, process optimisation can be carried out and specific safety constraints can be taken into account. A detailed study of the influence of food ingredients on both the inactivation and quality degradation kinetics is required to account for the complexity of food systems. Additionally, revisiting the mechanisms of the reactions of ozone with organic materials will contribute to establishing the impact of specific radical species on target microorganisms. Overall it can be concluded that ozonation is a potential treatment for producing safe and high-quality minimally processed fruit and vegetables but that specific treatment conditions must be developed and defined for each produce prior to ozone treatment.

References


