Decontamination of Raw Foods Using Ozone-Based Sanitization Techniques

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Abstract

Popular foods such as fresh produce and dry nuts are increasingly implicated in outbreaks of food-transmitted diseases. These products are not amenable to conventional processing technologies; therefore, many alternative decontamination methods are actively investigated. Ozone is a versatile sanitizer with promising applications in some high-risk foods. This antimicrobial agent is active against a broad spectrum of microorganisms, and it can be used effectively in its gaseous or aqueous state. The flexibility afforded by ozone use makes it a viable option for application on easy-to-damage products like fresh produce. If process parameters are adequately controlled, ozone treatment can enhance safety and increase shelf life without adversely affecting product quality. Despite these advantages, ozone may not be suitable for some applications, including treatment of liquid foods and products rich in unsaturated fats and soluble proteins. Ozone, as a powerful oxidizer, must be carefully controlled at all times, and equipment must be rigorously maintained to ensure safety of workers.

OZONE AND OTHER ANTIMICROBIALS: DEFINING THE TERMS

The term antimicrobials is used in many writings in reference to chemicals with a lethal or inhibitory effect against microorganisms. The term also is used broadly to refer to physical, chemical, or biological processes that are lethal to microorganisms or suppressive to their growth. Therefore, antimicrobials, as broadly defined, include (*a*) physical agents, e.g., heat or irradiation, (*b*) chemical agents, e.g., sanitizers or preservatives, and (*c*) biological agents, e.g., bacteriophage preparations that are proposed to control pathogenic bacteria in food. Some antimicrobials are indispensable elements of food safety assurance, whereas others have many uses in other fields such as medicine. Food processors use the term antimicrobials synonymously with preservatives, whereas in the medical field, it is used to refer to antimicrobial drugs. The great interest in discovering and testing new antimicrobials is driven by their significant impact on human health and economy. Therefore, new chemicals with potent antimicrobial properties are continuously sought.

Chemical antimicrobials may be classified on the basis of structure (e.g., phenolics or halogens), mode of action (e.g., oxidants or alkylating agents), targeted organisms, efficacy, application, or combinations of these factors. When a target organism is the main consideration, these antimicrobials are classified into antibiotics, antifungals, antiprotozoals, and antivirals. Based on application, the antimicrobial may be described as a sanitizer, disinfectant, antiseptic, or sterilant. This last grouping is the most practical; however, it is applied mainly to the antimicrobials used in vitro. Chemical sterilants (e.g., hydrogen peroxide vapor) are used to destroy all viable organisms on an object (e.g., food packaging material). Disinfectants commonly refer to chemicals applied topically to kill or inhibit pathogenic organisms on objects where the use of sterilants is impractical (e.g., floors or tables). A disinfectant or an antiseptic is applied to accomplish similar goals except that the latter is used on living tissues (e.g., wounds) and thus should be sufficiently nontoxic.

Differences between sanitization and disinfection are sometimes subtle. However, disinfection implies that the treated matrix is expected to be infectious, where as sanitization may serve as a precautionary measure on matrices that are not often contaminated with infectious agents. Thus, disinfection is a higher level of sanitization. The term sanitizer traditionally refers to antimicrobial chemicals used to decontaminate food-contact surfaces. If selected and used properly, sanitizers destroy vegetative cells of microorganisms of public health significance and substantially inactivate other undesirable microorganisms without adversely affecting the quality of the product or the safety of the consumer (Code Fed. Regul. 2009). The United States Food and Drug Administration (FDA) makes a distinction between a sanitizer and a disinfectant on the basis of the need to rinse the antimicrobial off food-contact surfaces (US Food Drug Adm. 1993). Approved sanitizers in the United States are those that do not require a rinse after the sanitization step; these include household bleach and quaternary ammonium compounds.

A given antimicrobial agent, such as ozone, may belong to more than one group if it has diverse applications. The FDA approved ozone use in food as an antimicrobial additive (Code Fed. Regul. 2001). According to a United States Environmental Protection Agency (EPA) fact sheet, treatment of waste water with ozone gas is described as disinfection (US Env. Prot. Agency 1999). Furthermore, use of aqueous ozone to rinse food or food-contact surfaces may be considered sanitization. Some of these diverse applications are covered in this chapter, but most of the attention is given to ozone as a sanitizer in food processing.

GENERAL CHARACTERISTICS

Ozone is a triatomic oxygen molecule arranged to form an obtuse angle (Horvath et al. 1985). The compound is liquid above approximately 80 K (Brown et al. 1955, Jenkins & DiPaolo 1956)

and boils at 161 K (Horvath et al. 1985). The reduction potential of ozone is 2.07 V, qualifying it as one of the strongest known oxidizers. In the gaseous state, ozone is denser than air (Horvath et al. 1985) and colorless at lower concentrations. It possesses a distinct odor described alternately as fresh or fishy, which is detectable by humans at concentrations as low as 0.02 ppm (Horvath et al. 1985). Under natural circumstances, small amounts of ozone are generated in the earth's atmosphere by the action of short-wave ultraviolet (UV) light (<300 nm) on molecular oxygen. It has been noted that the formation of stratospheric ozone confers a benefit to the biosphere by absorbing a considerable amount of UV light in the range that is most damaging to proteins and nucleic acids (Horvath et al. 1985). Ozone is extremely reactive, with a half life in the gaseous phase of approximately 12 hours (Horvath et al. 1985); in water, half life is reduced to only 20 to 30 minutes (depending on several factors, including water source, purity, temperature, etc.) (Kim et al. 2003). Because it is capable of reacting with a number of substances, including metals and organic compounds, the stability of ozone is greatly dependant on the materials used to contain it, the presence of organic contaminants, and other factors, including temperature and pH (with decreasing stability at increased temperatures and pH) (Kim 1998).

OZONE PRODUCTION

As a consequence of its reactivity, ozone cannot be stored for significant periods of time; therefore, it must be generated as needed. Ozone gas can be purposely generated using a number of methods. These include photochemical procedures, which employ UV light but generally result in low ozone concentrations, electrolysis of water to produce ozone and hydrogen gas, and corona discharge. Corona discharge is the most common method in use and is capable of producing relatively high concentrations of ozone. In this method, gas (air or dry oxygen) is passed between two electrodes separated by a dielectric material and a high energy discharge splits molecular oxygen into its atomic form. Atomic oxygen spontaneously combines with molecular oxygen to form triatomic ozone (Horvath et al. 1985). When oxygen is used as a feed gas, as opposed to air, higher levels of ozone are subsequently produced. Once produced, ozone can be used in the gaseous state or sparged into water to produce aqueous ozone for rinsing and washing applications.

OZONE DECOMPOSITION

The decomposition of ozone yields a number of oxidative radicals, including the superoxide anion radical and hydroperoxide radical, which subsequently gives rise to the hydroxyl radical. The hydroxyl radical is incredibly reactive, and much of the antimicrobial activity of ozone has been attributed to the subsequent reaction of its decomposition products. Radical reactions continuously self propagate until a quencher or inhibitor is encountered, at which point reaction ceases. Because radicals are known to react very quickly, efficacy of ozone diminishes when target microorganisms are surrounded with oxidizeable substrates.

OZONE MEASUREMENT

The high redox potential of ozone often allows for its use in small amounts and contributes to its rapid decomposition during application. The combination of these factors makes accurate measurement of ozone levels particularly difficult. As with its generation, there are several methods available for the determination of ozone concentration. Historically, the most reliable and widely used method for the determination of ozone in the aqueous phase has been the indigo method. This procedure is based on spectrophotometric determination of the decolorization of indigo

trisulfonate upon its reaction with ozone (Bader & Hoigne 1981). Ozone acts by disrupting the sole carbon-carbon double bond of the indigo reagent. The indigo method is accurate within 2% (Grunwell et al. 1983) and is still often used to measure ozone in the aqueous phase. For quantification of gaseous ozone, the most common and trusted method is based on UV-light spectrometry. Absorbance of UV at 254 nm corresponds to ozone concentration in the gas sample (Dunlea et al. 2006). Many commercially available ozone monitors use this technology, which is appropriate for a wide range of ozone concentrations and allows continuous, near real-time quantification of ozone residuals.

SAFETY CONSIDERATIONS

Due to the strong oxidizing power of ozone, there are several considerations that must be taken into account to ensure its safe utilization. Human exposure to ozone above certain levels can lead to a number of negative health effects. At low concentrations, ozone is a respiratory irritant that can cause headaches, coughing, dizziness, and nausea. Exposure for long periods of time or to higher levels of ozone (6 ppm) can lead to pulmonary edema. In this case, inflammation causes obstruction to the entry of alveoli and/or reduction in alveolar volume, leading to diminished breathing capacity (Horvath et al. 1985). Repeated exposures to ozone can result in permanent lung damage (Scheel et al. 1959). The respiratory system is the primary site of action in humans, and other effects, including vision loss, have been reported as well (Lagerwerff 1963). Standards set forth by the Occupational Safety and Health Administration (OSHA) of the United States specify that workers may not be exposed to concentrations exceeding 0.1 ppm for extended periods of time, or 0.2 ppm for short term exposure (US Dep. Labor, Occup. Saf. Health Adm. 2004). In order to avoid inadvertent exposure, material selection and equipment maintenance are particularly important. Ozone reacts with several commonly used materials, including rubber and plastic. Surfaces exposed to ozone should consist only of compatible materials. This issue has been discussed previously in published literature (Kim et al. 2003).

Although the presence of ozone is expected in the Earth's stratosphere, it is considered a pollutant when found in the troposphere. Ozone is produced in the troposphere by the interaction of sunlight and volatile organic compounds or nitrogen oxides. Levels of tropospheric ozone vary depending on time of day, season, and location, with daily and annual peaks generally observed during the sunniest part of the day (Heagle 1989) and in the spring months (Vingarzan 2004). Common levels of tropospheric ozone have been reported to fall in the range of 20 to 250 ppb, depending upon the preceding factors (Heagle 1989, Sanderman 1996), representing an increase of at least 100% during the past century (Vingarzan 2004). The increase has been commonly attributed to the rising use of automobiles in this time period.

Rising levels of ozone in the troposphere have raised concerns for a number of reasons, particularly the implications of exposure to plant and human health. Elevated ozone levels have been demonstrated to reduce crop yield by more than ten percent (Heagle 1989), and to make plants more susceptible to subsequent stressors (Sanderman 1996). Ozone pollution has also been linked to damage of coniferous trees in the Northern Hemisphere. Deleterious effects on human health have long focused on the induction of respiratory distress, but elevated ozone levels can also precede vasoconstriction, causing a rise in blood pressure (Brook et al. 2002), and have been linked to increased risk of myocardial infarction (Ruidavets et al. 2005).

ANTIMICROBIAL ACTION

Ozone possesses a wide antimicrobial spectrum. Its efficacy has been demonstrated against both Gram-positive and Gram-negative bacteria (Ingram and Haines 1949, Guzel-Seydim et al. 2004),

bacterial spores (Ishizaki et al. 1986, Khadre & Yousef 2001), fungi (Palou et al. 2001, Allen et al. 2003, Oztekin et al. 2006), viruses (Kim et al. 1980, Roy et al. 1981), and protozoa (Khalifa et al. 2001). Sensitivity of diverse microorganisms to ozone suggests that cells possess several sites for ozone action that leads to lethality. Early studies hypothesized that reaction of ozone with enzymatic systems interfered with cellular respiration (Ingram & Haines 1949). More recently, the focus has been on the interaction of ozone with the unsaturated lipids of the cell membrane (Victorin 1992, Thanomsub et al. 2002). Membrane damage induced by exposure to ozone has been demonstrated to result in leakage of cellular components followed by cell death (Scott & Lesher 1963). Membrane damage also allows ozone to penetrate into the cell, where it has been reported to cause DNA-strand breaks (Ishizaki et al. 1987). Damage to nucleic acids has been suggested to be a cause of viral inactivation by ozone (Kim et al. 1980, Roy et al. 1981). In bacterial spores, significant damage to spore coat has been observed (Khadre & Yousef 2001). A point of contention among researchers is whether the effects observed are attributable to the reactions of molecular ozone itself or to the decomposition products that are produced by its reversion to molecular oxygen. Among the products produced is the hydroxyl radical, the strongest known oxidizer, which leads many authors to believe that decomposition products are responsible for the antimicrobial effects (Block 2001). However, in a study conducted by Hunt & Marinas (1997), radical scavenging compounds were added to treatment media, and this addition did not have a significant effect on the reduction of *Escherichia coli* population by ozone treatment. This finding suggests that molecular ozone may play a significant role in bacterial inactivation. However, in a study conducted on *Bacillus subtilis* spores, hydroxyl radicals were found to be principally responsible for inactivation of these spores (Cho et al. 2002).

Several factors can alter the efficacy of ozone against microorganisms. The medium in which microorganisms are suspended or embedded plays a very important role in determining ozone's antimicrobial efficacy. Most significantly, the presence of organic material, especially proteins and fats, greatly reduces efficacy of ozone (Ingram & Haines 1949, Guzel-Seydim et al. 2004). As previously indicated, ozone stability decreases when medium pH increases. If molecular ozone reactions are necessary for inactivation, a low pH is desired; however, higher pH has been demonstrated to encourage formation of hydroxyl radicals, contributing to spore inactivation (Cho et al. 2002). Increased moisture also seems to enhance killing by gaseous ozone. This effect was observed in the treatment of barley grains to inactivate fungi (Allen et al. 2003). A similar study was conducted on wheat, wherein the authors concluded that increased water activity and increased treatment temperature (from 10°C to 40°C) led to greater inactivation of fungal spores (Wu et al. 2006). Increased relative humidity has been demonstrated to aid in bacterial spore inactivation (Ishizaki et al. 1986).

METHODS OF APPLYING ANTIMICROBIALS: GASEOUS VERSUS AQUEOUS STATE

Antimicrobial gases vary considerably in water solubility. Gases with low solubility may be mixed with water under pressure until being applied directly to the treated matrix (e.g., food). Gases that are readily soluble in water are well suited to aqueous applications. Food processors often contemplate the merits of applying antimicrobials as gaseous versus aqueous phases. In food processing, aqueous or gaseous sanitization should be a carefully designed unit operation. Aqueous sanitization is a standalone unit operation; however, this step is often combined, or directly preceded with a cleaning operation. Gaseous sanitization can be combined with many other unit operations such as transportation or refrigerated storage. This offers food processors a flexibility that is not

attainable in aqueous sanitizing operations. Schematics and photographs of pilot-scale aqueous and gaseous ozone treatment setups are displayed in **Figures 1** and **2**.

Regardless of water solubility, it may be preferable to apply antimicrobials in the gaseous rather than aqueous state. By their nature, gases diffuse faster than liquids and thus reach target microorganisms in the food more quickly, often within short treatment time. Additionally, applied gases are less likely to modify the composition of a treated matrix (e.g., its water and water-soluble contents). Simpler devices are generally used for treating a matrix with gases than with other forms of antimicrobials. However, antimicrobial gases are often toxic or explosive, and thus it is crucial to contain these gases during and after the treatment. Some antimicrobials, including ozone, have applications in both gaseous and aqueous states.

GASEOUS OZONE APPLICATIONS: EFFICACY AND CHALLENGES

Antimicrobial efficacy of gaseous treatments depends on the properties of the applied gas, properties of the treated matrix, and treatment conditions. Gases vary considerably in biocidal properties, which depend on the physical and chemical characteristics of the gas. A reactive gas with small molecular mass and good miscibility with water is likely more biocidal than gases with opposite characteristics. A matrix with a relatively smooth surface is likely less protective to exterior contaminants than one with a rough or porous surface. For example, it was easier to decontaminate an apple's smooth exterior surface than its rough surfaces at calyex and stem regions, when ozone gas was bubbled into wash water (Achen & Yousef 2001). Additionally, components of the food matrix may compete with contaminating microorganisms for applied gases.

Treatment conditions influence greatly the efficacy of biocidal gases. Concentration, time, temperature, and relative humidity are important parameters that should be watched carefully during treatment of food with antimicrobial gases (Vurma et al. 2009). Concentration of the antimicrobial gas in the treatment environment and time of exposure of the matrix to the gas define the treatment dosage. It is generally accepted that humidity is essential for reactivity of biocidal gases with treated microorganisms. Antimicrobials may have to pass from the gaseous to the aqueous phase to be effective against targeted microorganisms. Contribution of treatment temperature to the antimicrobial efficacy of biocidal gases is difficult to assess. Gases are more soluble in the aqueous phase of the food matrix at colder temperatures than at warmer temperatures, but reactivity of the gas with microorganisms should increase with temperature.

Treatment of food with antimicrobial gases has many challenges. Monitoring the sanitizer concentration is easier in aqueous than gaseous phases. In fact, there is no simple technique to compare the sanitizing efficacy of different gases (Hill 1905, Osipyan & Uspenskiy 1964). It is not surprising that aqueous sanitization is more developed and is applied more often, particularly in food, than are gaseous treatments. However, the recent increase in disease transmission by fresh produce is making it urgent to search for alternatives to conventional aqueous sanitization procedures. Gaseous decontamination of food, particularly fresh produce, is gaining interest in the food industry.

Ozone is applied as an antimicrobial agent in the gaseous or aqueous state. The gas has low water solubility; therefore, application of the agent in aqueous solution requires specialized equipment and well-trained operators. Low concentrations of ozone are used to decontaminate drinking water. Bottled water, for example, is treated so that residual ozone at the time of bottling does not exceed 0.4 mg liter^{-1} (Code Fed. Regul. 2006). Recently, moderate levels of gaseous ozone have been recommended in sanitization of fresh produce (Vurma et al. 2009). High ozone concentrations (~11% ozone in oxygen, wt/wt) have been tested successfully for decontamination of shell eggs (Rodriguez-Romo & Yousef 2005, Perry et al. 2008).

SELECTED FOOD APPLICATIONS

Fresh Produce

Compared with other food industries, the fresh produce sector likely will benefit the most from the recent advances in ozone sanitization technology. Application of gaseous and aqueous ozone in fresh produce have been explored by several researchers and contemplated by some processors, but major implementations have not yet materialized. The following discussion covers emerging safety concerns about fresh produce and recent developments in ozone sanitization technology. This discussion may help processors reevaluate the feasibility of applying ozone in the decontamination of fresh produce.

Safety of fresh produce and the need for alternative sanitizers. The nature of fresh produce is such that this category of foods presents a unique challenge in terms of quality and safety. Fresh produce is expected to reach the consumer in the raw state, and many fruit and vegetable tissues are highly susceptible to damage. These factors limit rigorous processing of fresh produce; consequently, these products often have a short shelf life and a relatively poor microbial safety record. With the increased sales of fresh produce in recent years, disease outbreaks associated with this category of foods are on the rise.

To further complicate this situation, contaminants commonly associated with fresh produce include not only pathogenic bacteria, but also viruses, such as Norwalk and hepatitis A, and parasites, most notably *Cryptosporidium* and *Cyclospora*. In addition to pathogens, produce is also highly susceptible to fungal spoilage. Not only does this lead to significant monetary losses for producers, but the presence of some fungi is a potential health hazard due to the production of mycotoxins. Some *Penicillium* spp., commonly responsible for blue mold rot on the surface of various fruit crops, are known producers of the mycotoxin patulin. Chiefly associated with apple products, the toxicity of patulin to animals has been documented (Becci et al. 2006).

Opportunities for exposure to microbes are numerous in the fruit and vegetable production chains. Given that fruits and vegetables must be grown in soil, microbes from this source are plentiful on these products. The nature of the environment in which fresh produce is commonly grown precludes complete control over presence of animals, especially birds and rodents. Fecal contamination of fresh produce due to the presence of these animals is not uncommon. Fertilizers and irrigation water are other possible sources of microbial contaminants. The widely spread 2008 outbreak of salmonellosis was attributed to contaminated water used on tomatoes, peppers, and cilantro (Cent. Dis. Control Prev. 2008). The use of improperly composted manure has caused contamination of produce on more than one occasion. This is a particular concern in the production of organic produce, which prohibits the use of chemical fertilizers. Due to the sensitive nature of produce, many types are harvested by hand, presenting an additional opportunity for contamination due to the poor hygiene practiced by some pickers.

Conventional processing of fresh produce begins with removal of field heat. In its most basic incarnation, this is accomplished by moving harvested product quickly from the field to refrigerated storage, but the process may be sped up using forced-air cooling, immersion in ice water, or even application of a vacuum, depending on the particular commodity and facility capabilities. If possible, products are washed by spraying or immersion. This process is useful for the removal of soil and foreign contaminants, but with the addition of a sanitizing agent, most commonly chlorine, washing can be used to reduce levels of surface microbiota. Chlorine possesses a wide antimicrobial spectrum and has a long history of use; however, increasing attention is being given to the generation of potentially harmful byproducts of this sanitizer.

The use of ozone as a sanitizing agent presents numerous advantages over traditional methods. It has been demonstrated that ozone works well against pathogenic bacteria (including bacterial spores), viruses, parasites, and fungi at relatively low concentrations (Kim et al. 2003). The efficacy of ozone in both aqueous and gaseous phases allows it to be used on a wide variety of products. The antimicrobial efficacy of ozone may depend on the generation of reactive oxygen species (e.g., hydroxyl radicals), but these are short-lived byproducts that do not remain in treated food until the time of consumption. Therefore, the generation of toxic byproducts during sanitization is not a concern when using ozone, which decomposes to harmless molecular oxygen. Unlike chlorine, ozone has been defined as a suitable additive for organic products, making it one of few sanitizers available for use in this growing category of foods. The following sections provide a summary of current research regarding the use of ozone to enhance safety and quality of fresh produce.

Ozone in fresh produce processing. Ozone, applied in either the aqueous or gaseous states, has been investigated as a sanitizer for a number of produce commodities against several target pathogens. These treatments have been particularly successful on products with a smooth outer surface, where contaminants are easily accessible by the sanitizer. Das et al. (2006) reported complete inactivation of spot-inoculated *Salmonella* Enteritidis on cherry tomatoes treated with 20 mg liter^{-1} gaseous ozone for 15 min. Although this treatment resulted in color loss of treated product, lower levels of gaseous ozone (4 μl liter^{-1} for 30 min, repeated treatment) have been used successfully without producing this negative effect (Aguayo et al. 2006). Tomatoes subjected to this treatment displayed markedly slower softening of flesh (Aguayo et al. 2006), a finding that was also reported in kiwi fruits (Li et al. 2009) and has been attributed to inactivation of fruit pectin methylesterase, an enzyme involved in the degradation of pectin (Rodoni et al. 2010). Another possible fringe benefit of ozone treatment is increased accumulation of phenolic compounds. Enhanced production of these compounds has been demonstrated in tomatoes and grapes following ozone treatments (Artes-Hernandez et al. 2007, Rodoni et al. 2010). Treatment of apples with 23–30 mg liter^{-1} aqueous ozone resulted in a 3.7-log reduction of *E. coli* O157:H7, but reduction in the stem and calyx regions of the fruit was drastically less, not even one log (Achen & Yousef 2001). This difference aptly highlights the importance of the product's surface texture and accessibility of ozone to entrapped contaminants on sanitization efficacy.

Cantaloupe melons have repeatedly been the cause of outbreaks of salmonellosis due to the transfer of contaminants on the rind to cut fruit. In studies conducted by Selma et al. (2006, 2008a), whole melons were treated with ozone gas, hot water, or a combination of the two treatments. Immersion in 75°C water followed by treatment with 10,000-ppm gaseous ozone for 30 min resulted in a 3.8-log reduction of mesophilic bacteria and 2.1-log reduction of coliforms. In a study using precut melon cubes (2008b), this group reported a lack of adverse sensorial effects after treatment with 20,000-ppm gaseous ozone for 30 min, demonstrating the promise of ozone use to improve melon safety. Fruit juices have also been treated with ozone. Apple cider has previously been implicated in outbreaks of *E. coli* O157:H7 infections. In 2004, Williams and colleagues reported a 6-log reduction in this pathogen after 45 min of ozone addition (9 g h^{-1}) to apple cider held at 50°C. The same conditions produced a 4.8-log reduction of *Salmonella* in 30 minutes.

Many researchers have investigated the possibility of ozone use to sanitize leafy greens. Interest in leafy green safety has increased conspicuously since 2006, when baby spinach was linked to an outbreak in the United States of *E. coli* O157:H7, which sickened more than 200 people. In work with shredded lettuce, Kim et al. (1999) reported a 1.9-log reduction in total count after three minutes of treatment with 1.3 mM aqueous ozone. Treatment with 5-ppm aqueous ozone for five minutes was reported to decrease the counts of *Shigella sonnei* by 1.8 log (Selma et al. 2007).

Yuk et al. (2007) inoculated enoki mushroom with *E. coli* O157:H7 and *Listeria monocytogenes* and treated the inoculated product (without agitation) for 5 min with 3-ppm ozone solution. This treatment decreased the populations of these pathogens by only 0.94 and 0.34 log, respectively. Klockow & Keener (2009) developed a process that involves the generation of ozone within spinach packaging. Although this technique resulted in 3- to 5-log inactivation of *E. coli* O157:H7, the authors reported significant quality deterioration of the treated product. In a 2009 study, Vurma and colleagues addressed the possibility of integrating ozone into existing spinach processing. They applied gaseous ozone (1.5 g O_3 kg^{-1} gas mixture or 935 ppm v ozone/v gas mixture) during vacuum cooling to inactivate up to 1.8 log of *E. coli* O157:H7 without obvious quality loss (**Figure 3*a*,*b***). Low-level ozone treatment (5 to 10 ppm) during simulated transportation resulted in a 1-log inactivation, and the combination of these procedures yielded ≥4-log reduction of this pathogen (Vurma et al. 2009). The processing of leafy greens, clearly a promising application for ozone technology, illustrates another commodity-based consideration. Because of the delicate nature of leaves, product damage must be avoided when designing a potential treatment. More intensive treatments were investigated in the research discussed earlier, but product damage was significant enough to preclude their use (**Figure 3*c***).

Concern regarding fungal spoilage prevents fresh berries from being washed between harvest and market. Gaseous ozone has been investigated for use in berries both to eliminate pathogens and to extend product shelf life. Treatment of blueberries with 5% (wt/wt) gaseous ozone for 64 min resulted in a 2.2-log reduction of *E. coli* O157:H7; similar conditions under pressure inactivated 3 log of *Salmonella* (Bialka et al. 2007). Despite the high levels of ozone and long treatment time utilized in this study, no color loss or other negative sensorial effects were reported. In an attempt to extend shelf life of strawberries, 0.35-ppm gaseous ozone was maintained during refrigerated storage for three days. Authors reported a slight decrease in incidence of gray mold after two days of storage, but no difference between treated and untreated strawberries was observed after four days (Perez et al. 1999). Experiments by another researcher involved storing strawberries at 25°C in an environment containing a mixture of ozone (10 ppm, v ozone/v gas mixture) and carbon dioxide (Vurma 2009). The author reported rapid quality deterioration of untreated strawberries, compared to ozone-treated product (**Figure 4**). The ozone treatment provided up to an eight-day extension of shelf life when compared to untreated berries.

Delayed appearance of fungal growth has been observed in a number of ozone-treated commodities. Palou et al. (2001) reported a one-week extension of the shelf life of oranges stored under 0.3-ppm ozone for four weeks. Even after mold growth occurred, authors reported a significant decrease in sporulation of *Penicillium* spp. with continuous exposure to ozone, speculating that this effect may prevent the spread of fungal contamination on fruit during storage. In a study utilizing different types of produce, Tzortakis et al. (2008) reported suppression of fungal spore formation ranging from 20% (on plums) to 95% (on clementines) after storage in the presence of 0.1M ozone for 13 days. Fungal sporulation was prevented during four weeks of storage in the presence of 0.3-ppm ozone on peaches inoculated with *Monilinia fructicola*, *Botrytis cinerea*, *Mucor piriformis*, and *Penicillium expansum* (Palou et al. 2002). No injury to fruit was observed, and respiration and ethylene production were unaltered during subsequent ripening in ambient atmosphere.

Success in ozone treatment of fresh produce is not limited to laboratory and pilot-scale operations. A treatment involving 15- to 30-second exposure to 300-ppm gaseous ozone followed by extended storage in the presence of approximately 1-ppm ozone has been successful commercially in preventing the spread of fungal disease on onions and potatoes (Rice 2006). Producers implementing this system were able to increase yields of marketable product enough to recover the cost of equipment investment in the first growing season of use. A fresh produce processor in Tennessee integrated an ozone rinse into an existing production line and now uses ozone in

conjunction with a subsequent application of lower concentrations of chlorine. This change has led to a nine-day increase in the shelf life of bagged salads and has significantly decreased water usage at the plant (Rice 2006).

The examples discussed earlier address a wide variety of commodities and treatments. The suitability of ozone treatment for a particular product depends largely on the product's susceptibility to damage during ozonation. Optimization of ozone concentration, treatment time, and phase of application can lead to favorable outcomes in the majority of commodities. There are however, several examples that illustrate the unsuitability of ozone for certain applications where ozone treatment does not achieve the desired reduction in microbial population or the expected improvement in product quality. Studies on alfalfa seeds and sprouts have demonstrated relatively low lethality toward pathogens inoculated on these products. A 64-min treatment consisting of constant sparging of ozone into water (initial concentration of 21 $\mu g\ ml^{-1}$) resulted in a 2.2-log reduction of *E. coli* O157:H7 on alfalfa seeds (Sharma et al. 2002). Similar treatments on alfalfa sprouts yielded a 2-log reduction of the same pathogen, and application of pressure did not increase lethality (Sharma et al. 2003). In treatments targeting *L. monocytogenes*, sparging of seeds in ozonated water (21.3 $\mu g\ ml^{-1}$) reduced pathogen population by less than 1.5 log and caused significant damage to seeds (Wade et al. 2003). Soaking of sprouts in ozonated water (20 $\mu g\ ml^{-1}$) for up to 20 min resulted in a 1.68-log reduction of aerobic microbes, but only 0.94-log reduction of *L. monocytogenes*, and significantly reduced the sensory quality of sprouts (Wade et al. 2003).

A series of studies conducted by Tiwari and associates investigated the effect of ozone treatment on anthocyanins and ascorbic acid in various fruit juices. Work performed on blackberry juice demonstrated significant decreases in ascorbic acid and anthocyanin content. Degradation of both compounds was correlated with both ozone concentration and treatment time (Tiwari et al. 2009a). Similar results were confirmed in strawberry and grape juices (Tiwari et al. 2009b, 2009c). On the contrary, treatments of fresh cut celery with up to 0.18-ppm aqueous ozone resulted in no appreciable difference in ascorbic acid content (Zhang et al. 2005), and treatment of whole blackberries with 0.3-ppm gaseous ozone did not elicit loss of either color or anthocyanins (Barth et al. 1995). Celery and blackberries did, however, display a significant decrease in activity of polyphenol oxidase and peroxidase, respectively, a positive outcome due to the quality deterioration associated with these enzymes.

Dried Foods

Dried foods are not generally susceptible to bacterial contamination due to their low water activity. This same characteristic, however, makes microbiota quite difficult to inactivate. Additionally, dry products are often heavily laden with fungal and bacterial spores. Many of these spores are resistant to heat, acid, and other antimicrobial treatments, making it extremely difficult to reduce these populations with minimal processing. Fumigation of such dried products has traditionally employed methyl bromide. However, owing to the fact that this chemical is an ozone layer–depleting substance, its use has been prohibited in recent years, leading to increased use of phosphine gas.

When Oztekin et al. (2006) treated dried figs with gaseous ozone (up to 10 ppm) for 5 h, they observed less than 1-log reduction of total aerobic and fungal counts. Coliform bacteria were more sensitive to this treatment; an initial population of 1.46 $\log\ g^{-1}$ was reduced to undetectable levels after three hours of exposure to 3-ppm ozone (Oztekin et al. 2006). A subsequent study compared gaseous and aqueous ozone treatments. Aqueous ozone (1.7 $mg\ liter^{-1}$) was more effective than the gaseous treatment (13.8 $mg\ liter^{-1}$) at reducing coliforms and yeast populations, but both treatments reduced native populations of *E. coli* and molds to below detection limit after 15 min of treatment (Zorlugenc et al. 2008). In a study on dates, coliforms and *Staphylococcus aureus* were both

inactivated by more than 3 log after a 60-minute treatment with 5-ppm gaseous ozone (Habibi Najafi & Haddad Khodaparast 2009). Treatment of red pepper flakes with 9-ppm gaseous ozone for 360 min provided a 1.5-log reduction of *Bacillus cereus* spores but also reduced consumer scores for color and flavor (Akbas & Ozdemir 2008).

In 2003, Allen and colleagues reported that treatment of barley with gaseous ozone (0.16 mg g^{-1} min^{-1}, 5 min) resulted in greater than l-log reduction of fungal spores. The authors also reported that inactivation was positively correlated with increased water activity of the product and treatment temperature. Treatments of up to 0.98 mg g^{-1} min^{-1} ozone for 45 min had no detrimental effect on barley germination (Allen et al. 2003). Similar results were obtained in a subsequent study of stored wheat (Wu et al. 2006).

Insect infestation is a problem unique to stored products including grains. Reports of insect resistance to the most widely used grain fumigant, phosphine, have been widespread in recent years (Chaudhry 1997). In a study of stored maize, treatment with 50-ppm gaseous ozone for three days resulted in greater than 90% mortality of three common stored grain pests (Kells et al. 2001).

Animal Products

Animal products receive a great deal of attention among food safety specialists because of their association with well-known pathogens (i.e., enterohemorrhagic *E. coli* with beef and *Salmonella* with poultry). A recent large-scale outbreak of salmonellosis due to consumption of contaminated eggs (U.S. Cent. Dis. Cont. Prev. 2010) emphasizes the need for measures to control or eliminate pathogenic microorganisms in these products.

Meat. Undercooked meat is a common cause of foodborne illness, which makes it a high profile target for safety enhancing treatments. Unfortunately, success with ozone treatment of meat products has been limited. Reductions of target populations are typically low because ozone is consumed by reacting with organic compounds covering the surface of the meat, which, apart from decreasing antimicrobial activity, often also decreases the quality of the final product. This effect is illustrated well in a 1979 publication by Yang & Chen in which bacterial suspensions were made from spoiled poultry meat. Treatment with 19 mg $liter^{-1}$ aqueous ozone reduced an initial count of over 7 log per ml to undetectable levels, but addition of egg albumen significantly reduced the biocidal effect of ozone due to increased ozone demand of the medium (Yang & Chen 1979). In a study of beef prior to grinding, meat was treated with 1% aqueous ozone for up to 15 min. At the maximum treatment time, reductions in *E. coli*, *Salmonella* Typhimurium, coliforms, and aerobic plate count were all less than 1 log (Stivarius et al. 2002). A subsequent study utilizing 1% aqueous ozone for 15 min with successive treatments with either cetylpyridinium chloride or acetic acid produced slightly more inactivation, but all reductions were still less than 2 log. Additionally, treatments with ozone and either cetylpyridinium chloride or acetic acid decreased the red color of the meat, and the treatment utilizing acetic acid was reported to produce off odors (Pohlman et al. 2002). Gaseous ozone (0.03 ppm) was used to treat beef sides during dry aging, but treated samples displayed significantly more discoloration and shrinkage than controls, with no resulting shelf life increase of steaks (Greer & Jones 1989). Aqueous ozone (5 ppm) had limited lethality against *Clostridium perfringens* vegetative cells and spores on fabricated beef surfaces (Novak & Yuan 2004). Inactivation of the vegetative cells was enhanced when the ozone treatment was followed by heating at 45°C or 55°C for 30 min. Similarly, inactivation of *C. perfringens* spores increased when the ozone treatment was followed by a 30-min heating at 55°C or 75°C.

Chicken breasts were inoculated with *Salmonella* Infantis or *Pseudomonas aeruginosa* and treated with >2000 ppm gaseous ozone for up to 30 min. Reduction of these organisms was less than 2 log, and no reduction of native coliforms was detected (Al-Haddad et al. 2005).

Seafood. Multiple studies investigating the use of ozone in shrimp farming operations have shown the promise of this application. In these studies, ozone is introduced into hatchery tanks until a maximum residual ozone level is reached. Residual concentrations of 0.35 mg liter^{-1}, maintained for 30 min have been demonstrated to reduce levels of the pathogen *Vibrio harveyi* by approximately 3 log (Meunpol et al. 2003). Adolescent shrimp are not harmed by low-level ozone treatment, and the reduction in pathogens affected by these treatments results in greater survival of shrimp with reduced administration of antibiotics (Blogoslawski et al. 1992, Meunpol et al. 2003).

Ozone treatment (0.20 mg liter^{-1}) of water containing fish pathogens, including *Aeromonas*, *Yersinia*, and *Vibrio* spp., as well as infectious pancreatic necrosis virus, decreased their level by up to 4 log, regardless of water salinity (Liltved et al. 1995). However, modest results were observed when ozone was tested in aquaculture systems. Low-level ozone treatment (0.039 kg ozone kg^{-1} feed) reduced outbreaks of bacterial gill disease in rainbow trout, despite the observations that pathogen reduction in water was less than 1 log, and bacterial colonization of gills was not prevented (Bullock et al. 1997). Additionally, ozone toxicity to fish was an intermittent problem and has been observed in several species (Bullock et al. 1997, Summerfelt & Hochheimer 1997). Toxicity remains the main obstacle for the use of ozone in these applications.

Although use of ozone in fish farming is challenging, application of the sanitizer to extend the shelf life of whole or filleted fish seems promising. Low-level ozone treatment on fresh scad filets did not provide significant reductions in populations of inoculated microorganisms, but storage in the presence of ozone (0.25 mg liter^{-1}) increased the lag phase of several populations to five or more days (Da Silva et al. 1998). Treatment of oysters with 5 μg liter^{-1} ozonated water for 2 min resulted in a less than 1-log decrease of indigenous microbiota. A combination treatment utilizing ozone and chitosan provided a similar initial reduction, but extended the lag phase to ten days and increased the shelf life of the product from 8 to 20 days (Rong et al. 2010).

When whole fresh megrim were washed in 2-ppm aqueous ozone and subsequently stored for 12 days in ice made from ozonated water, total microbial counts on treated fish remained low enough for this product to be sold in the European Union, whereas untreated fish had to be discarded (Pastoriza et al. 2008). Additionally, sensory analysis indicated that ozone-treated fish stored for 3 to 11 days was preferred to untreated fish in both the raw and cooked states. In studies examining the storage of sardines in slurry ice (a mixture of salt water and ice crystals), the addition of ozone to the mixture (0.17 mg liter^{-1}) led to a shelf life increase of 3 to 4 days as well as improved sensory outcomes. The presence of ozone during storage in slurry ice (for up to 22 days) kept the levels of sardines' natural microbiota significantly lower than those in samples not subjected to ozone; ozone treatment was not associated with increased lipid oxidation (Losada et al. 2004, Campos et al. 2005). Similar results were obtained in a subsequent study on farmed turbot, with a shelf life extension of seven days (Campos et al. 2006). Despite the modest lethality reported in the previous studies, ozone delayed proliferation of microbial population during storage of these products. Treatments like these could minimize economic losses associated with spoilage of seafood.

Shell eggs. Several researchers have investigated the use of ozone to increase the safety of shell eggs. In the US, all liquid egg products are pasteurized, but no such treatment is required for shell eggs. It was estimated that 1 in 20,000 eggs produced in the United States is contaminated internally with *Salmonella* Enteritidis; however, many more may carry this pathogen on the

shell (Musgrove et al. 2005; U.S. Dep. Agric., Food Safety Inspec. Serv. 2005). In a 2000 study, Koidis and associates dipped inoculated shell eggs into ozonated water (3.0 mg liter^{-1}) for 30 to 90 sec; this treatment decreased *Salmonella* Enteritidis population by less than 1.5 log per egg. Rodriguez-Romo & Yousef (2005) treated externally contaminated eggs with gaseous ozone, UV radiation, or a combination of the two steps. Treatment with gaseous ozone (5% by weight, 5 in lb^{-2} gauge) for 8 min decreased *Salmonella* Enteritidis by 2.6 log per gram of egg contents. Treatment with UV radiation (254 nm, 100 μw cm^{-2}) for 4 min resulted in a reduction of 3.8 log, but the combination of these technologies provided a reduction of 4.6 log in only 2 min of treatment time (Rodriguez-Romo & Yousef 2005). Subsequent investigation regarding the use of gaseous ozone against *Salmonella* Enteritidis inside shell eggs has led to the development of a process combining sequential application of mild heat and gaseous ozone under pressure to provide >6 log inactivation. This process is effective against *Salmonella* located in the egg yolk and produces eggs similar in quality to untreated eggs (Perry & Yousef 2010).

Potential Control of Toxins and Pesticide Residue

Presence of mold on grains, nuts, and some fruits is associated with the contamination of these products with mycotoxins. One of the mycotoxins is aflatoxin, a secondary metabolite of *Aspergillus* spp., occurring in four forms (B_1, B_2, G_1, and G_2). Presence of aflatoxin in food is heavily regulated due to the fact that this fungal metabolite is highly toxic. Aflatoxin B_1 is also a potent carcinogen. Due to these health risks, aflatoxin levels in foods are capped in the ppb range, varying slightly depending on the product. Contamination with aflatoxin leads to significant economic losses for producers of grains and nuts. Various experiments have been undertaken to assess the effect of ozone on aflatoxin. Short gaseous ozone treatments (2% by weight for 15 seconds) have reduced the toxicity of several mycotoxins, including aflatoxin, ochratoxin, and patulin suspended in liquid media (McKenzie et al. 1997). Although aflatoxins B_1 and G_1 were degraded by 2% ozone treatment for 5 min, aflatoxins B_2 and G_2 required treatment with 20% ozone. The relative susceptibility of toxins B_1 and G_1 has previously been reported in a study using peanut and cottonseed meal (Dwarakanath et al. 1968). More recently, corn kernels contaminated with aflatoxin were treated with gaseous ozone at 10% to 12% (wt/wt) for 96 hours. This treatment resulted in a 92% reduction of aflatoxin levels (Prudente & King 2002). When dried figs were spiked with aflatoxin B_1, treatment with gaseous ozone (13.8 mg liter^{-1}) for 180 min reduced the toxin level by more than 95% (Zorlugenc et al. 2008). In a 1997 study, it was reported that fifteen seconds of treatment with 10% ozone gas (wt/wt) reduced patulin in aqueous solution to undetectable levels and eliminated its toxicity (McKenzie et al. 1997). A more recent study reinforced the efficacy of ozone against patulin in diluted apple juice (Cataldo 2008).

In recent years, the presence of pesticide residue on fresh produce has become a source of alarm to many consumers. Consumers concern has contributed significantly to the increase in sales of organic products, especially in the fresh produce category, in the United States. Ozone can be used to sanitize organic products, and some researchers suggest the treatment can be useful in decreasing pesticide residue. Significant research has been conducted regarding the ability of ozone to reduce pesticide levels in drinking water (reviewed by Ikehata & El-Din 2005). Studies investigating degradation of pesticide residue on food products are few, but the results of such studies are promising. In a study utilizing pak choi spiked with different pesticides, degradation in aqueous solution was significantly greater than degradation on vegetable tissues (Wu et al. 2007). However, treatment with 2 mg liter^{-1} ozonated water for 30 min resulted in significantly greater removal of cypermethrin (61%), methyl-parathion (48%), parathion (54%), and diazinon (53%) than washing with tap water alone, which only resulted in 27% to 31% removal of these

compounds (Wu et al. 2007). Washing apples with ozonated water (3 ppm) reduced commonly applied levels of mancozeb and ethylenethiourea to unquantifiable levels (Hwang et al. 2006). The ability of ozone to combat pathogens, mycotoxins, and chemical contaminants simultaneously is a benefit not offered by other treatments.

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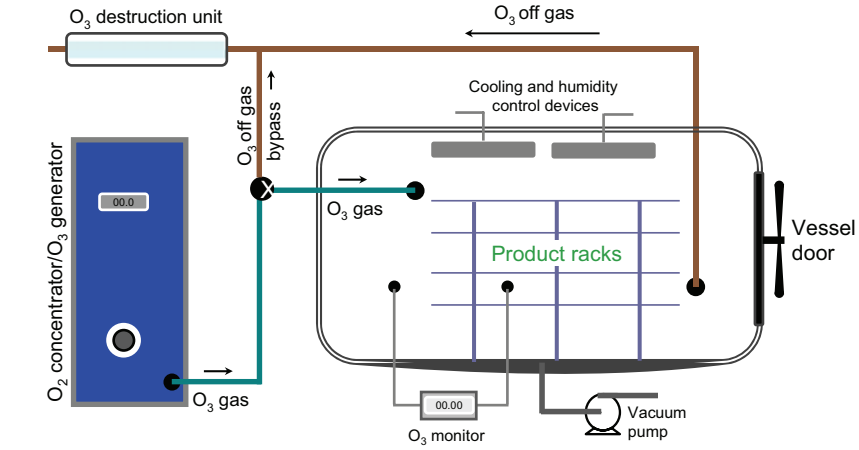

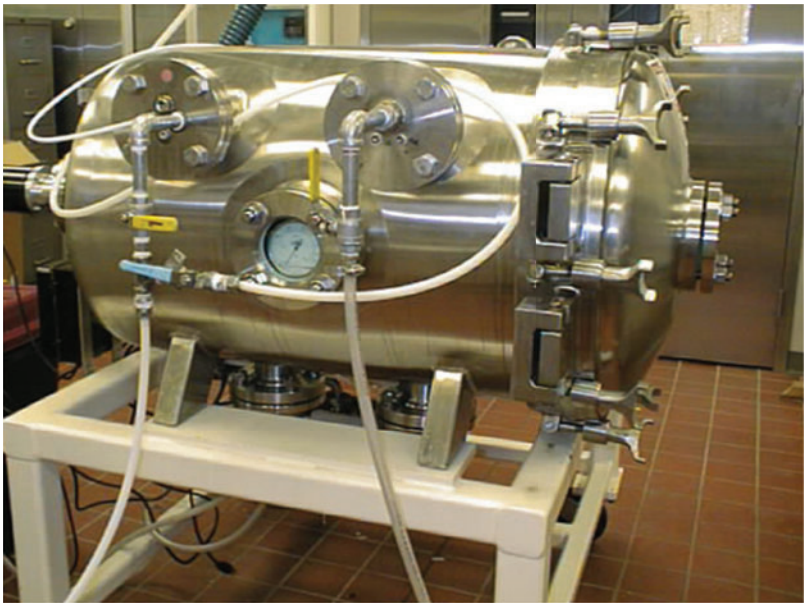

Figure 1

Prototype of a gaseous ozone system for decontaminating raw food products; equipment is set up and operational at the author's laboratory.

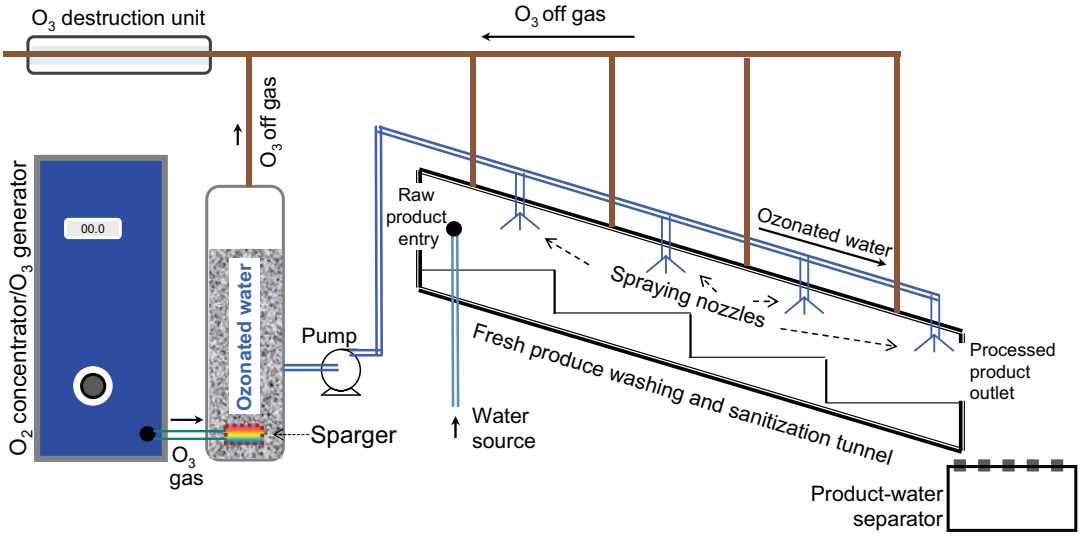

Figure 2

Prototype of an aqueous ozone system for application in fresh produce washing and sanitizing; equipment is set up and operational at the author's laboratory.

Figure 3

Baby spinach samples that were untreated or treated, during vacuum cooling, with gaseous ozone (1.5 g O_3 kg^{-1} gas mixture or 935 ppm v ozone/v gas mixture) followed by pressurization at 10 psig for different holding times to eliminate 1.8 log *Escherichia coli* O157:H7 (Vurma et al. 2009; pictures are courtesy of M. Vurma). (*a*) Untreated; (*b*) treated for 30 min, product quality comparable to the untreated control; (*c*) treated for 45 min, product quality deterioration is noticeable.

Figure 4

Strawberries that were untreated or treated with gaseous ozone/carbon dioxide mixture and held at 20°C for up to 3 days. Treatment involved subjecting the berries to an environment containing16 mg ozone kg^{-1} gas mixture (10 ppm v ozone/v gas mixture) for 4 hours (Vurma 2009). (*a*) Untreated at time zero; (*b*) untreated and stored for 3 days, mold was noticeable; (*c*) treated and stored for 3 days, berries remained mold-free.

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Errata

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