Postharvest Use of Ozone on Fresh Fruit

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In 1997, an expert panel reviewed the safety and potential for food processing use of ozone and declared ozone to be Generally Recognized As Safe (GRAS) for food contact applications (U.S. FDA 1997). Their declaration of GRAS status for ozone was submitted to the Food and Drug Administration and its use on food products is legal in the United States (Rice 1999). Since that time, interest in developing ozone applications in the food industry has increased. In the mid-1990's, ozone was approved for food processing in Japan, France, and Australia. Most recent regulatory actions have primarily addressed ozone applications in water. Ozone has been reviewed for water disinfection applications (Nickols and Varas 1992; White 1992; Rice 1999), for food processing applications (Graham et al, 1997), its chemistry has been described (Razumoffski and Zaikov, 1984), and the practical aspects of the design and operation of ozonators have been reviewed (Rice and Netzer, 1984).

Ozone in Air of Storage Rooms

Ozone in air has also received considerable research (EPRI Expert Panel 1997) and commercial interest recently. Both benefit (Jin et al, 1989; Liew and Prange, 1994; Pinilla, et al, 1996) and lack of benefit (Hopkins and Loucks, 1949; Spalding 1966, Spalding 1968) of ozone in air use in fruit and vegetable storage rooms have been reported repeatedly. Ozone in air at concentrations that can be breathed over long periods without irritation cannot be expected to provide effective sanitation of fruit and vegetable surfaces or storage rooms. The application of ozone in air concentrations that effectively kill pathogen spores exceeds 0.1 ppm (the exposure limit for workers from US OSHA) and therefore requires that measures to protect workers be employed. The active compounds produced by ozone in air generators are not clear at this time, because some produce more than ozone. Some function by scrubbing ethylene and spores from an air-stream that passes through the device, so the ozone concentration in the storage room air is not elevated. Ethylene destruction in air by ozone in air is a well-documented phenomena (Dickson, et al, 1992), and for those commodities that benefit by its removal, ozone may be of use, assuming the fruit are not injured by the gas. We will emphasize ozone in water applications in this article because many aspects of ozone use in air have been documented, and more research is currently in progress at the F. Gordon Mitchell Postharvest Center of the UC Kearney Agricultural Center that will be presented at a later date.

Ozone in Water Systems

Ozone in water is often described as an alternative to hypochlorite as a disinfectant or sanitizer, although they differ in many aspects (Table 1). Ozone solubility in water is low, its maximum solubility at 20°C(68°F) is 29.9 µg/ml; in practice, it is difficult to exceed 10 µg/ml, and many systems produce 5 µg/ml or less. Ozone in water above 1 µg/ml can liberate ozone into the air that exceeds safe levels (OSHA workplace maximum = 0.1 ppm). Significant advantages of ozone in water are that it decomposes quickly to oxygen, leaving no residues, and it has more potency against bacteria, cysts of protozoa, viruses, and fungal spores than hypochlorite (White 1992). Ozone was reported to have a mode of action to control a plant pathogen not based solely on its antimicrobial activity. Sarig et al (1996) reported ozone controlled *Rhizopus stolonifer* and induced resveratrol and pterostilbene phytoalexins in table grapes, and that these made the berries more resistant to subsequent infection. Ozone can oxidize many organic compounds, particularly those with phenolic rings or unsaturated bonds in their structure (Razumovski and Zaikov 1984) and can have a role in reducing pesticide residues in process water (Nickols and Varas 1992) and mycotoxins in durable commodities (McKenzie, et al 1997).

Some packinghouse processes where ozone in water could be applied include:

1. **Ozonation to santitize packingline process water.** The water in tanks where fresh fruit are dumped or floated before cleaning, sorting, and packing operations is an important site for the accumulation of pathogens that infect fruit later in storage, shipping, or marketing. Examples are blue mold of apples and pears, caused by

Table 1. *Comparison of various aspects of hypochlorite and ozone use in water*

Attribute	Hypochlorite	Ozone
Microbial potency	Kills plant pathogens and microbial saprophytes effectively. Some human-pathogenic, spore-forming protozoa resistant. Maximum allowable rates under regulatory control	Kills plant pathogens and microbial saprophytes effectively, including spore-forming protozoa. Maximum rate limited by ozone solubility, difficult to exceed about 10 µg/ml
Cost	Chemical cost low. Repeated delivery required, sometimes pH and concentration controller systems needed, minor maintenance and energy costs, chlorine storage issues	Variable: no chemical cost, but high initial capital cost for generator, usually needs filtration system when water re-used some are complex, modest maintenance and energy costs
Influence of pH	Efficacy diminishes as pH increases, above pH 8, pH adjustment may be needed. Chlorine gas released at very low pH (4 or less)	Potency not influenced very much by pH, but ozone decomposition increases at high pH
Disinfection by-products	Some regulatory concern, tri-halo compounds, particularly chloroform, of some human safety concern	Less regulatory concern, small increase in aldehydes, ketones, alcohols, and carboxylic acids created from organics, BrO3- from OBr-
Worker safety issues	Chloroamines can form and produce an irritating vapor, chlorine gas systems require on-site safety measures, OSHA (TWA) limit for chlorine gas: 1 µg/ml	Off-gas ozone from solutions an irritant and must be managed. MnO_2 ozone destruction efficient and long-lived. OSHA (TWA) limit for ozone gas: 0.1 µg/ml
Persistence in water	Persists hours in clean water, reduced persistence to minutes in dirty water	Persists minutes in clean water, reduced persistence to seconds in dirty water
Use rates	Limited by regulation to 25 to 600 µg/ml, depending on application	Not limited by regulation, but Henry's law limits theoretical maximum ozone in water to about 30 µg/ml at 20°C (68°F). Most ozone systems produce 5 µg/ml or less.
Use in warm water	Increases potency, some increase in vapors	Not practical, rapidly accelerates ozone decomposition, increases off-gassing, decreases ozone solubility
Influence on product quality	Little risk of injury at recommended rates. Some injury possible above 50 µg/ml on tree fruits. Off-flavors on some products at high rates	In brief water applications, risk of product injury low. Stem, calyx, and leaf tissue more sensitive than fruits. Risk of injury needs more evaluation.
Impact on water quality	Minor negative impact: water salt concentration increases somewhat, may interfere with fermentation used to reduce Biological Oxygen Demand, some pesticides inactivated, discharge water dechlorination may be required.	Mostly positive impact: does not increase salt in water, many pesticides decomposed, Biological/Chemical Oxygen Demand may be reduced, flocculation and biodegradability of many organic compounds enhanced, precipitates iron, removes color, odors
Corrosiveness	High, particularly iron and mild steel damaged	Higher, particularly rubber, some plastics, yellow metals, aluminum, iron, zinc, and mild steel corroded

Penicillium expansum, and green mold of citrus, caused by *Penicillium digitatum*. Therefore, disinfection of this water is important, and usually is accomplished with hypochlorite. Ozone has been employed in flume water in apple and pear packinghouses, and some facilities have ozonated hydrocooler water. Pre-conditioning of the water (to reduce particulates, BOD, turbidity, etc.) before ozonation is needed in systems where water is recycled, and this can be difficult and expensive. A contact time of two minutes in 1.5 µg/ml ozone killed 95-100% of all eight fungi tested, and none survived 3 minutes of contact (Figure 1). Spores of these pathogens die quickly in ozonated water, but fruit, soil, and other debris in the water can reduce the ozone concentration completely or to ineffective low levels.

2. **Ozone in water treatment of pathogens inoculated into wounds on fruit.** Many pathogens use wounds on the fruit surface, that usually occur at harvest, to initiate infections that are visible days afterward. These infections are typically controlled by fungicides which are applied on fruit packing lines, an example is green mold of citrus, caused by *Penicillium digitatum*. In our tests with citrus fruit, ozone in water has not been effective for this application, and there are no reports where application of ozone in water for this purpose has been successful on other fruit. Disease control efficacy of ozone cannot be predicted by toxicity of ozone to pathogens in water. The control of pathogens inoculated into wounds on fruit, a common mode of infection for the spores of many fungi, fails even after prolonged treatment with very high ozone concentrations in water, although the spores are killed very quickly in ozonated water (Figure 1). Pathogens are even more protected from ozone than microbes that reside on the product surface, presumably because of reduced ozone penetration into the wounds, the leakage of ozone-reactive substances that reduced ozone dosage inside the wounds, or antioxidants that protected the spores. In tests with citrus fruit, the incidence of green mold on oranges, lemons, and grapefruit inoculated with spores of *Penicillium digitatum* and treated with water alone or water with 12 µg/ml ozone for 5 minutes at 20°C (68°F; pH 7.2) was 100%. Similarly, the incidence of sour rot on oranges and grapefruit inoculated with spores of *Geotrichum citri-aurantii* and treated with water alone for 5 minutes was 54%, while the sour rot incidence among those treated for 5 min with 12 µg/ml ozone for 5 minutes was 78%. Similar results were obtained with lemons, even when the ozone contact period was increased to 20 minutes. The inability to control infections on inoculated citrus fruit with ozone treatment in our tests agrees with the results of Spotts and Cervantes (1992) in their work with ozone in water treatment of pears. Like ozone, hypochlorite was similarly ineffective for the control of pathogens in wounds in our tests. Similarly, prior work with hypochlorite and chlorine dioxide at practical concentrations (200 µg/ml or less) showed they did not control infections within inoculated wounds on citrus (Eckert and Eaks 1989; Smilanick et al 1999; Smilanick, unpublished) or pear (Spotts and Peters 1980) fruit.

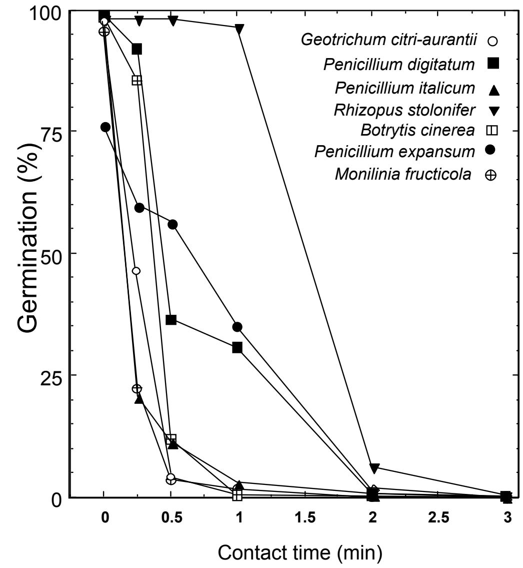

Figure 1. *Germination of spores of various postharvest pathogenic fungi after exposure to 1.5 µg/ml ozone in water at 16.5°C (62°F) and pH 6.4.*

3. **Treatment of fruit with ozone in water to control pathogens on the fruit surface before they initiate infections.** These pathogens can be controlled by fungicide or sanitizer applications, an example is the contamination of grapes by spores of *Botrytis cinerea*, cause of gray mold. Immersion in 10 µg/ml ozonated water for 1 to 4 minutes reduced gray mold about 50% on table grapes in recent tests. Ozone effectiveness was irregular and dependent on grape berry condition. We suspect that cracks on the berry

surface or around the pedicle of the berries may have protected spores from the ozone in trials where its efficacy was poor. It was not superior to 200 µg/ml hypochlorite for 1 minute for this purpose. This fungus can also infect grapes through wounds or by latent infections from the field, which probably would not be controlled by ozone in water or hypochlorite treatment.

4. **Ozone in water treatment to reduce natural microbe populations on fresh fruit.** The quality of some products is reduced when natural microbe populations on them are high, although most are saprophytes that do not cause postharvest decay or comprise a food safety hazard. The few reports describing this application report population reductions of 90-99% by immersion of fresh vegetables or fruit in ozonated water for 2 minutes or more (USFDA 1997). The reductions in part are due to washing the product alone with sterilized water, and not the direct action of ozone on the product surface. In our work, immersion of strawberries in ozone at 4 µg/ml for 2 minutes reduced aerobic mesophilic bacteria populations by 92.3% and yeast and mold populations by 91.0%.

5. **Ozone treatment for water quality purposes.** Some facilities have water quality discharge compliance issues that could be alleviated by ozone treatment. These include remediation of: 1) pesticide residues; 2) organic compounds [defined by biological/biochemical (BOD) or chemical (COD) oxygen demands] or; 3) suspended solids, because ozone facilitates their flocculation and precipitation. Increases in the salt content of water can also be avoided because hypochlorite salts are no longer used. We added 44.8 g, 20.3 g, and 83.3 g of the postharvest fungicides imazalil, thiabendazole, and sodium ortho-phenyl phenate, respectively, to a 2000 L-capacity ozone in water system. Imazalil, thiabendazole, and sodium ortho-phenyl phenate were measured at 17.5, 6.4, and 8.6 µg/ml, respectively, in the water before ozonation; these rates simulated their concentration in discharge water from a packinghouse. The water temperature was 18.4°C (65°F) and the pH was 6.1. After mixing for one hour without ozone, the ozonator started, and subsequent samples were taken at 0.5, 1, 2, 4, and 8 hours. More than 95% of all three fungicides were destroyed within 30 minutes. In a test with strawberry wash water, we observed reductions in aerobic mesophilic bacteria populations, suspended solids, BOD, and COD of 99.2%, 43%, 33%, and 43%, respectively, after two hours of operation with 4 µg/ml ozone in a 2000 L-capacity ozone in water system.

Ozone is a sanitizer that can minimize chemical and microbial contamination of water within dump-tanks, floatation solutions, brush bed or high pressure washers, or other process water that contacts the fruit during postharvest handling. Conditioning of water before ozonation will be needed in most applications. Sanitation of fruit surfaces can be achieved, but contact times must be long, compared to other sanitizers, and the ozone concentration must be high (>1 g/ml). It could replace hypochlorite for the control of gray mold, but probably with some loss in efficacy. Ozone is compatible with bicarbonate salts. Immersion in sodium bicarbonate solutions comprise an inexpensive and effective treatment for postharvest sour rot and green mold of citrus. Ozone could increase the life of bicarbonate solutions, by reducing BOD/COD and clarifying the solution, and it would kill nuisance microbes that contaminate bicarbonate solutions that accumulate with repeated use. Ozone can also have a role in reducing fungicide residues in discharge water; this aspect could be a benefit in some situations.

More research to assess the benefits of ozone in water treatment on other commodities, such as peaches, plums, and nectarines, should be conducted. Sarig et al (1996) reported that ozone controlled *Rhizopus stolonifer* and induced resveratrol and pterostilbene phytoalexins in table grapes, making the berries resistant to subsequent infection. Because this research showed ozone may have a mode of action not based solely on its antimicrobial activity, empirical testing employing the treatment of inoculated fruit should be done to assess its efficacy.

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