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PACKINGHOUSE NEWSLETTER

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Postharvest Use of Ozone on Citrus Fruit

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Introduction. Interest in ozone applications for agriculture and food processing has increased in recent years. Ozone has a long history as a water disinfectant and is in common use for this purpose in many parts of the world. In the mid-1990s, ozone was approved for food processing in Japan, France, and Australia. In 2001, ozone was declared a GRAS (generally recognized as safe) substance by the FDA and the USDA has since approved its use on meats and on certified organic foods. Many aspects of ozone use have been reviewed in the literature: 1) water disinfection applications (Nickols and Varas 1992; White 1999; Rice 1999); 2) food safety and sanitation (Graham et al. 1997; Kim et al., 1999), 3) chemistry (Razumovski and Zaikov, 1984), 4) responses of horticultural products to ozone (Forney, 2003); and 5) the practical aspects of the design and operation of ozonators have been reviewed (Rice and Netzer, 1984). However, many applications have yet to be developed, and there are large gaps in our knowledge about where ozone could be used in packinghouses.

Ozone in air

Like thiabendazole or imazalil, ozone gas retards the production of fungal spores on infected citrus fruit at relatively low ozone concentrations of 0.3 to 1.0 ppm (µL/L; Harding 1968; Palou et al 2002; 2003). Spore production is only retarded when the gas is present and resumes when the fruit are removed from the ozone atmosphere. Inhibition of spore production is valuable because without spore production, the infection cycle is broken. In some packinghouses (especially those that store fruit over long periods), many of the green mold spores may be resistant to the common fungicides. Resistant fungi produce millions of spores even when exposed to fungicide and lead to the further build-up of resistant spores and fruit decay. In these cases, ozone could retard the production of these spores when no other method is available.

Sporulation control with ozone has been successful when the fruit are in cold storage (50°F or less); one manager reported ozone did not retard sporulation effectively at 68°F (20^{o}C). A basic issue is ozone penetration into fruit containers. Ozone penetration into most conventional citrus packages is poor with adequate penetration only occurring in packages with large vents or open tops, such as bins (Palou et al 2003). Palou et al (2003) showed in a room containing 0.7 ppm (µL/L) ozone, penetration into returnable plastic containers with large vents was good. The ozone concentration inside them was 0.6 ppm (µL/L), and good sporulation control was evident. However, penetration to oranges inside plastic bags or fiberboard cartons was 0.1 ppm (µL/L) or less and sporulation was not controlled.

Sanitation of equipment and fruit surfaces with ozone gas has been attempted. However, very high ozone concentrations are required to kill pathogenic fungi within a few hours or days and these require corrosion-resistant facilities that contain the gas, enhanced safety measures, and presumably other measures to scrub ozone from vented air. To kill spores of the pathogens that cause green mold, blue mold, and sour rot (*Penicillium digitatum, P. italicum*, and *Geotrichum citri-auranatii*, respectively) in humid air (~95% RH) at 41^{o}F (5°C) within one hour, we found about 200 ppm (µL/L) ozone was required. If the air was dry (35% RH), a dose 5 to 10 times higher was required.

The rapid reaction of ethylene and ozone in air is a well-documented phenomenon (Dickson, et al, 1992), and for those commodities that benefit by ethylene removal, ozone may be of use, assuming the fruit are not injured by the gas. However, the benefits of reducing ethylene to very low levels during citrus fruit storage have not been established. Some devices function by passing ethylene and spore-laden air from the storage room through the device so that ozone concentrations within the storage room are not elevated. Oxidation products other than ozone, particularly oxides of nitrogen, can be emitted if the air that passes into a corona discharge ozone generator is not dry. Such additional products may have some affect on the fruit and possibly reduce decay (Jin et al., 1989). We and others have observed that citrus fruit tolerate ozone gas at concentrations much higher than those that harm other produce, but few studies on this subject have been done. Garcia and coworkers (1998) reported that storage of three navel orange (Lanelate, Navelate and Salustiana) and two mandarin (Fortune and Ortanique) varieties at 41^{o}F (5°C) in 0.1 ppm (µL/L) ozone did not affect quality parameters such as juice content, soluble solids content, pH, and titratable acidity values of citrus fruits during shelf life. Color development was delayed among fruit stored in the ozone atmosphere, which could be a benefit. However, the incidence of oleocellosis among Lanelate and Navelate oranges in the ozone atmosphere was higher.

Equipment that can safely and quickly deliver doses of ozone gas as high as 10,000 ppm (µL/L) or more, under a partial vacuum, was recently developed by PureOx Co. (Sparks, NV). It is being investigated as a possible replacement for methyl bromide fumigation to control insects and microorganisms.

Ozone in Water

Ozonation to sanitize packingline process water. The water in tanks where fresh fruit are dumped or floated before cleaning, sorting, and packing is an important site for the accumulation of pathogens. Fruit passing through this water is easily infected causing decay in storage or during shipping and marketing. Therefore, disinfection of this water is important and usually is accomplished with hypochlorite (chlorine). 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 has been used to sanitize flume water in apple and pear packinghouses and some facilities have ozonated hydrocooler water. 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, 1999). Spores are killed with relatively small doses. A contact time of two minutes in 1.5 ppm (µg/mL) ozone killed 95-100% of the spores of eight fungi we tested, and none survived 3 minutes of contact (Figure 1). Furthermore, ozone can oxidize many organic compounds and can therefore have a role in reducing pesticide residues in process and discharge water (Nickols and Varas 1992). In tests we conducted, more than 95% of imazalil, thiabendazole, and sodium ortho-phenyl phenate (OPP) in water were destroyed within 30 minutes. These attributes make ozone a good choice when processed water is recycled.

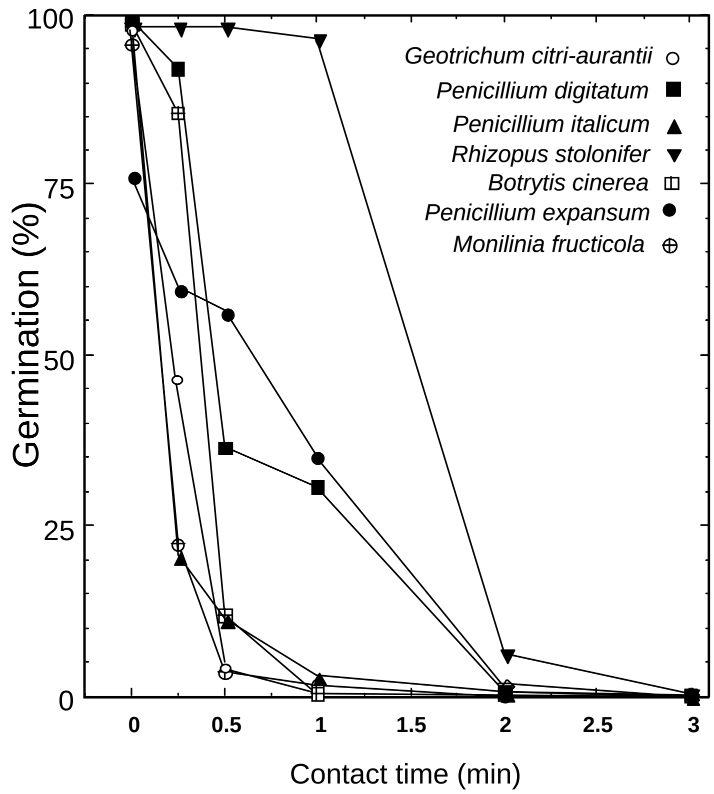

Figure 1. Germination of spores of various postharvest pathogenic fungi after exposure to 1.5 ppm (µg/mL) ozone in water at 62°F (16.5°C) and pH 6.4.

Note that the term "ppm" is used both for water concentrations of ozone, where the unit is weight/volume (µg/mL), and gas concentrations, were ppm is a volume/volume unit (µL/L). Thus, 1 ppm (µL/L) of ozone in air contains only 1/500,000 as many molecules of ozone as 1 ppm (µg/L) in water.

Some sanitation of the surface of fruit can be achieved, but most of the cleaning effect we have seen with ozonated water sprayed over brushes was modest and only slightly better than washing with sterile water alone. Typical reductions in Penicillium spore or natural microbe populations were 90 to 99% (1 to 2 $\log_{10}$). Unlike chlorine, ozone does not tolerate the addition of surfactants or heat to improve its effectiveness.

Ozone in water is a dissolved gas and its solubility is relatively low; the maximum concentration is about 30 ppm (µg/mL) at 68°F, and it readily off-gases. Ozone in water above 1 ppm (µg/mL) can liberate ozone into the air that exceeds safe levels, particularly if the packinghouse is warm and the water passes through high pressure, small-droplet size nozzles. In addition, the presence of organic and other ozone-reactive compounds in the water or from soil or fruit constituents can quickly react with ozone and cause the concentration to plummet. In practice, even with clean water, it is difficult to exceed 10 ppm (µg/mL), and many systems produce 5 ppm (µg/mL) or less. Pre-conditioning (e.g. flocculation and filtration) of the water to reduce particulates, organic compounds, turbidity, etc. is needed before ozonation in systems where water is recycled or the source water is of poor quality.

Ozone in water to control pathogens entering through wounds. Many citrus pathogens (e.g., green mold and sour rot) invade fruit tissue through wounds received during harvest or subsequent handling. These infections are typically controlled by postharvest fungicide drenches or fungicide application during packingline operations. In our tests with citrus fruit, ozonated water has not been effective at reducing this type of decay , and there are no reports where it has been successful on other fruit. Although spores are killed very quickly in ozonated water, once pathogens have penetrated into fruit tissue they are protected and are not controlled even after prolonged treatment with very high ozone concentrations in water (Smilanick et al. 2002b). In tests with citrus fruit, the incidence of green mold on oranges, lemons, and grapefruit inoculated with spores of *P. digitatum* and treated with water alone or water with 12 ppm (µg/mL) ozone for 5 minutes at 68°F (20°C; pH 7.2) was 100%. The incidence of sour rot on oranges and grapefruit inoculated with spores of *G. 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 ppm (µg/mL) ozone was 78%. Similar results were obtained with lemons, even when the ozone contact period was increased to 20 minutes. The inability of ozone to control infections on inoculated citrus fruit agrees with similar work conducted on pears (Spotts and Cervantes, 1992).

Ozone does not differ from other sanitizers in its inability to stop wound pathogens. Hypochlorite and chlorine dioxide at practical concentrations (200 ppm or less) also showed no control of infections within inoculated wounds on citrus (Eckert and Eaks 1989; Smilanick et al 2002a) or pear (Spotts and Peters 1980) fruit.

Safety

Ozone is toxic and workers must be protected from it. The federal exposure limit in workplaces for ozone gas, a time-weighted average during an eight-hour workday, is 0.1 ppm (µL/L). The concentration that is "immediately dangerous to life or health" (IDLH) is 5 ppm (µL/L). This is the maximum concentration for which there are approved respirators; rates higher than 5 ppm are dangerous and require self-contained breathing equipment. To be in compliance with state and federal safety codes, the capability to determine ozone concentrations in air on-site is usually required.

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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 ppm (µg/mL).
Cost	Chemical cost low. Repeated delivery required. Sometimes pH and concentration controller systems needed. Minor maintenance and energy costs. Chlorine storage issues. Need water of at least moderate quality.	Variable: no chemical cost, but high initial capital cost for generator. Usually needs filtration system if water re-used. Generators are complex. Modest maintenance and energy costs. Must have high quality, clean water with low oxidation/reduction potential.
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 rapidly above pH 8.
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, bromate can form from bromine.
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 ppm (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 ppm (µL/L).
Persistence in water	Persists hours in clean water, persistence reduced to minutes in dirty water.	Persists minutes clean water, persistence reduced to seconds in dirty water.
Use rates	Limited by regulation to 25 to 600 ppm (µg/mL), depending on application.	Not limited by regulation, but Henry's law limits theoretical maximum ozone in water to about 30 ppm (µg/mL) at 68°F (20°C). Most ozone systems produce 5 ppm (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 of 200 ppm (µg/mL) or less.	In brief water and low concentration gas applications, risk of injury to citrus appears low, but 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.