RULES AND REGULATIONS
Federal Register
Vol. 66, No. 123
Tuesday, June 26, 2001

PART 173-SECONDARY DIRECT FOOD ADDITIVES PERMITTED IN FOOD FOR HUMAN CONSUMPTION

1. The authority citation for 21 CFR part 173 continues to read as follows:

Authority: 21 U.S.C. 321, 342, 348.

2. Section 173.368 is added to subpart D to read as follows:

Sec. 173.368 Ozone.

Ozone (CAS Reg. No. 10028-15-6) may be safely used in the treatment, storage, and processing of foods, including meat and poultry (unless such use is precluded by standards of identity in 9 CFR part 319), in accordance with the following prescribed conditions:

(a) The additive is an unstable, colorless gas with a pungent, characteristic odor, which occurs freely in nature. It is produced commercially by passing electrical discharges or ionizing radiation through air or oxygen.

(b) The additive is used as an antimicrobial agent as defined in Sec. 170.3(o)(2) of this chapter.

(c) The additive meets the specifications for ozone in the Food Chemicals Codex, 4th ed. (1996), p. 277, which is incorporated by reference. The Director of the Office of the Federal Register approves this incorporation by reference in accordance with 5 U.S.C. 552(a) and 1 CFR part 51. Copies are available from the National Academy Press, 2101 Constitution Ave. NW., Washington, DC 20055, or may be examined at the Office of Premarket Approval (HFS-200), Center for Food Safety and Applied Nutrition, Food and Drug Administration, 200 C St. SW., Washington, DC, and the Office of the Federal Register, 800 North Capitol St. NW., suite 700, Washington, DC.

(d) The additive is used in contact with food, including meat and poultry (unless such use is precluded by standards of identity in 9 CFR part 319 or 9 CFR part 381, subpart P), in the gaseous or aqueous phase in accordance with current industry standards of good manufacturing practice.

(e) When used on raw agricultural commodities, the use is consistent with section 201(q)(1)(B)(i) of the Federal Food, Drug, and Cosmetic Act (the act) and not applied for use under section 201(q)(1)(B)(i)(I), (q)(1)(B)(i)(II), or (q)(1)(B)(i)(III) of the act.

[66 FR 33830, June 26, 2001; 67 FR 271, Jan. 3, 2002]

Ozone in Food Processing Applications

Past Experience, Future Potential and Regulatory Issues:

Zentox Corporation

Abstract

The recent stories of food contamination reported in the media serve to highlight the need for the food industry in the United States to seek better, more effective methods of ensuring the safety of food products. In the vast majority of cases reported, the culprits have been identified as *Escherichia coli*, *Salmonella enteriditis*, *Listeria monocytogenes* and other pathogens found in fruits, meats, shell fish and other foods consumed by the public. The results of these cases of contamination have been serious illnesses and, in some
cases, fatalities.

The use of ozone in the processing of foods has recently come to the forefront as a result of the recent approval by the U.S. Food and Drug Administration approving the use of ozone as an anti-microbial agent for food treatment, storage and processing. The FDA approval marks a watershed event for the food industry. Prior to the approval, FDA had approved ozone for use only as a disinfection mechanism for bottled water production and the sterilization of bottled water lines. The recent regulatory breakthrough is a result of efforts made by the Electric Power Research Institute (EPRI) and the panel of technical experts assembled to review and evaluate the efficacy and safety of ozone in food processing.

It is worthy of noting that, the use of ozone in food processing has been allowed and accepted in Japan, Australia, France and other countries for some time. There is a plethora
of documentation and supporting literature attesting to the benefits of ozonation as a food product sterilization methodology some of which will be reviewed herein.

The FDAís recent approval serves to provide the basis for expanded use of ozone in food processing with application ranging from produce washing to recycling of poultry wash water to seafood sterilization. A sampling of these applications will be presented in the attached case studies.

The 1997 EPRI Expert Panel Report

This important reference document is organized as follows: An Executive Summary that is followed by chapters dealing with Efficacy of Ozone, Applications of Ozone, Safety Issues and Ozone Toxicology, Nutrient Impacts of Ozone and Summary Options of the Expert Panel. Also
within the EPRI document are various appendices containing the qualifications of the experts, abstracts and citations of pertinent articles dealing with the use of ozone in

processing and preserving foods. Specific applications are divided into 37 categories including; treatment, disinfection, eggs, fish, fruits, meats, poultry, vegetables, storage, etc.

Introduction

Ozone, first discovered in 1840 (Schonbein), began being utilized as a disinfection agent in
the production of potable water in France in the early 1900ís. The majority of early development was limited to Europe where it became more widely used in drinking water treatment. The potential utility of ozone to the food industry lies in the fact that ozone is 52% stronger than chlorine and has been shown to be effective over a much wider spectrum of microorganisms than chlorine and other disinfectants. Complementing the effectiveness, is the fact that ozone, unlike other disinfectants, leaves no chemical residual
and degrades to molecular oxygen upon reaction or natural degradation. The fact that ozone has a relatively short half-life is both an asset and a liability to practitioners. This is particularly true in treatment of drinking water where ozonation is employed to enhance filtration and provide primary disinfection but requires the addition of chlorine as the terminal disinfectant to maintain a residual in the distribution system.

Ozone is effective killing microorganisms through oxidation of their cell membranes and most of the pathogenic, foodborne microbes are susceptible to this oxidizing effect. During food processing operations, surface disinfection of the product (raw or partially processed) is very important. This is supported by the following statistics:

1. An estimated 30% of fresh produce is lost by microbial spoilage from the time of harvest, through handling, storage, processing, transportation, shelving and delivery to the
consumer (Beuchat 1991).

2. The USDA estimates the costs associated with foodborne illness to be in the range of $ 5.5 billion to $ 22 billion per year.

History of Early Ozone Applications in Food Processing:

Some early reported uses of ozone in food processing and preservation have been reported and documented. The examples below represent a sampling of notable cases:

1. Fish and Shellfish Preservation:

Washing of fish with ozonated water extended shelf life for 5 days:

Experiments performed by Violle in 1929 found that ozonated seawater spiked with various strains of bacteria (*B. typhus, B. coli,* etc.) resulted in sterilization that was comparable to what
was obtained in fresh water. Further experiments showed that exposure of shellfish to

ozonated water did not adversely affect the taste or appearance of the shellfish. Thus, Violle concluded that preozonation of water was suitable treatment for depuration of shellfish. Later work by Salmon and Le Gall (1936) built upon the work of Violle and reported that fresh fish, stored under ozonated ice, were edible for between 12 and 16 days. Fish treated with sterilized ice (presumably hypochlorous acid treated) were inedible after the 12th day and, possibly after the 8th day.

The original work in this area was carried further by others and reportedly resulted in the installation of an ozone system designed to sterilize 2000 kg per day in Le Havre and another facility in Boulogne-sur-Mer for treating a daily output of 6000 kg.

2. Ozone Used in Cold Storage for Meats:

Gaseous ozone was used as a preservation agent in meat and egg storage:

Kuprianoff (1953) reported that the first known use of ozone as a food preservation agent was in Cologne, Germany dating back to 1909. Later industrial applications using gas phase ozone for food preservation were reported in 1924 where Hartman stated that *ìin cold storage ozone is successfully used to prevent the growth of fungiî and ì eggs have been carried at a relative humidity of 88 and 90 percent and mold developments inhibited with the use of ozone*î. Hartman summarized by noting that ozone *ìhas manifold applications in cold storage and splendid results are being obtained in practice with this reagent every dayî.*

3. Disinfection and Preservation of Fruits and Vegetables:

One of the earliest reported experiments dealing with preservation of fruits was related to ozonation of bananas (Gane - 1933, 1934, 1935, 1936). Since then, numerous studies have been conducted on a wide variety of fruits and vegetables including carrots, broccoli,
pears, peaches and apples. The vast majority of these studies have reported some degree of shelf life extension and reduction of pathogenic contamination.

Recent Studies of Ozonation in Food Processing:

In spite of past regulatory restriction limiting usage of ozone in the U.S. food processing industry, numerous studies were undertaken to determine the efficacy and economics of ozonation. The examples below serve to illustrate some of these studies and conclusions.

1. Fruits and Vegetables:

Several controlled studies have been reported in the food science literature relating to the evaluation of treatment of fruits and vegetables with ozonated water. Kondo et al (1989) observed greater than 90% reduction of total bacterial counts upon treatment of Chinese cabbages with ozonated water (2.3 mg/L) for 60 minutes.

Treatment of wash water used in processing of carrots has been reported to provide 3 log reduction of bacteria (Williams et al 1995).

Barth et al (1995) evaluated ozone exposure for prevention of fungal decay on thornless blackberries. The fruit was harvested and stored for 12 days at 2 deg. C in 0.0, 0.1 and 0.3 ppm ozone, then evaluated for fungal decay (*Botrytis cinerea),* anthocyanines, color and peroxidase activity. Ozone storage suppressed fungal development for 12 days, while 20%
of control fruits showed decay. The treated fruit did not show observable injury or defects.
Sarig et al (1996) showed that ozone at low dosages (0.1 mg/g fruit) for 20 minutes, reduced the levels of fungi yeasts and bacteria on grapes, but that higher doses caused some fruit damage.

2. Poultry:

The poultry processing industry is a large volume consumer of water. The potential for reuse of poultry processing water represents an attractive economic benefit to the industry. In 1996 the USDA approved the use of ozonation for washing of poultry carcasses (provided that the ozone did not come into direct contact with the product). Studies by Chang and Sheldon (1989) reported that a combination of screening, diatomaceous earth filtration and ozonation yielded the highest quality of water with total microbial loads (total coliforms, *E. coli* and *salmonella* reduced by 99%). A subsequent study, Chang and Sheldon (1989) found no significant differences in measures of carcass quality including skin color, taste or shelf life using recycled, ozone treated water as opposed to fresh makeup water. The investigators further reported that a 2.7 log reduction in of total plate count was observed in the recycled water stream. The results of this study showed that, for a typical broiler processing plant (240,000 broilers/day) the savings resulting from chiller water reuse would equate to 50% reduction in discharges and savings of more than $ 45,000 per year.

3. Extension of Food Product Storage Life:

The following are a sampling of studies directed at application of gaseous ozone in food storage facilities.

A 1980 study (Gabrielíyantsí) showed that cheese stored with periodic ozonation prevented mold growth for 4 months while controls showed mold growth as soon as 1 month.

Japanese researchers indicated good results in the treatment of grains, flour and raw noodles with ozone with significant reductions in microbial growth.

Dondo et al (1992) reported that ozone treatment during refrigerated storage stabilized the surface bacteria count on beef and reduced that on fish.

Naitoh (1989) showed that ozone treatment inside a confectionery factory reduced airborne microorganisms over a 1 - 1.5 year period, ìremarkablyî inhibited bacterial growth and extended storage life of the product by 7 days.

4. Ozone Treatment of Dye Wastewater

Several studies have shown ozone and ozone/hydrogen peroxide to be effective in removal of color resulting from dye processing. Dyes used in food processing are typically easier to oxidize with ozone since they are organically based compounds.

Discussion of Current and Future Applications

Ozone is by no stretch a panacea or universal solution for all food processing operations. There are a significant number of good, sound applications including; disinfection of food wash water, wastewater treatment and recycling, treatment of cooling water and process water, and gas phase sterilization of products in storage and transport. Along with these beneficial applications comes some limitations. There are existing restrictions relating to human exposure to ozone (OSHA and EPA) which must be considered. Plant operators seeking to employ ozone will be faced with system design and process operation challenges.

Proliferation of ozone applications in the food industry is assured. As of this writing, the industry is free to start using ozone. Operators wishing to apply ozone are expected to employ Good Manufacturing Practices to protect workers from inadvertent exposure to ozone as well as to avoid overuse of ozone which may cause damage to the food product.

Conclusions

As a result of the recent FDA ruling, it is anticipated that ozone in food processing will be accelerated due to a number of factors:

1. The use of chlorine by the U.S. food industry is coming under increasing scrutiny by regulators due to toxicity issues and disinfection byproducts (DBPs).

2. Ozone has been proven to produce greater lethality rates for microorganisms than chlorine or other chemical sanitizing agents.

3. The recent surface water rules promulgated by the U.S. EPA relating to chlorine and chlorine derived DBPs will undoubtedly stimulate operators to seek technologies which will assure discharge compliance.

4. The increasing cost of water resources (both makeup and discharge), together

with the drive toward more conservation, will stimulate industry to seek treatment methods which allow recycling of product wash water and process water.

5. Advances in ozone generation and applications technologies have continued to make the process more reliable and economical.

Winegrowing
January/February 2000

Use of ozone for winery and environmental sanitation

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Since the early 1900s, ozone has been widely used for water treatment, including disinfection of municipal water supplies, swimming pools, spas, cooling towers, and sewage treatment plants. Recently, ozone has been used in food processing for sanitizing raw materials and irrigation waters, sanitizing packaging materials and storage facilities, and for sanitizing water for recycling.

Prior to 1997, ozone could only be used for sanitation and purification of bottled drinking water in the U.S., and it is widely used around the world for this purpose today. In May 1997, an expert panel assembled by the Electric Power Research Institute (EPRI) declared ozone to be Generally Recognized as Safe (GRAS) for use in food processing in the U.S.

Since then, wineries have embraced the use of ozone. Its use has been generally accepted and documented to be effective for barrel cleaning and sanitation, tank cleaning and sanitation, clean-in-place systems, and for general surface sanitation.

This same trend of acceptance has been noted in many other industries, such as fresh-cut produce processing; produce storage facilities; food processing, including meat processing facilities; and, as noted above, in the bottled water and beverage industries. In these industries, the ozone systems are generally permanently mounted or fixed in place, which makes management of off-gas and ozone monitoring for safety and efficacy relatively easy.

However, in the wine industry, ozone systems tend to be mobile, with multiple operators in multiple locations. This makes it important that safety features and ozone management systems be in place and that the system itself be reliable and easy to operate.

Understanding how ozone works

Ozone, or O_3, is generated in nature as a bluish or colorless gas characterized by the clean fresh smell in the air following a thunderstorm. When oxygen (O_2) and electricity interact, ozone is created, and this is why we smell ozone around copy machines, electric motors, or during arc welding.

Natural levels range from 0.01 ppm to 0.15 ppm and can reach higher concentrations in urban areas. Ozone is an unstable gas and readily reacts with organic substances. It sanitizes by interacting with microbial membranes and denaturating metabolic enzymes.

Ozone will also attack microbial biofilms and degrade them much as it would any other polysaccharide. Upon release of its oxidizing potential, ozone reverts back to oxygen from which it was generated. Application of ozone does not leave a chemical residual, and under ambient conditions, it has a half-life of 10 to 20 minutes. Thus, ozone must be electrically generated on-demand and cannot be stored for later use.

Ozone is generated by irradiation of an air stream with ultra-violet (UV) light at a wavelength of 185 nm or by passing dry air or oxygen through a corona discharge (CD technology) generator. For low ozone concentrations (ca. 0.14% by weight, or 0.5 grams per hour), the less expensive UV equipment is sufficient. For more demanding situations, where higher ozone concentrations (1.0% to 14% by weight) are required, CD systems are used.

The wine industry is using both CD technology and UV. Some manufacturers use multiple UV tubes to achieve a desired level of output. Several manufacturers chose to install air-cooled or water-cooled CD generators in their systems. It is really a question of how much ozone at a certain gpm is desired for an application. For CIP, 20 gpm may be desired, necessitating a larger system, while only 10 gpm at a lower concentration may provide satisfactory barrel washing.

Using ozone safely

Ozone is a toxic gas and must be monitored in the workplace when in use. However, in almost 100 years of industrial use, there has never been a human death attributed to overexposure to ozone. The Occupational Safety and Health Administration (OSHA) has set limits for ozone exposure in the workplace. These limits are for continuous eight-hour exposure of no more than 0.1 ppm, and a short-term exposure limit (STEL) of 15 minutes at 0.3 ppm, not to be exceeded more than twice per eight-hour work day.

Consequently, ozone requires monitoring in the workplace if used for environmental or equipment sanitation. Ozone monitors are readily available, and the supplier of ozone generating equipment should be able to assist with the selection and use of such monitors.

A manual containing all the relevant safety information for working with ozonating systems is essential; it should also contain operating instructions for the wineryÕs generating system. Workplace monitoring for ozone off-gas must be performed, and records must be maintained to assure OSHA compliance.

When ozone is generated, it is important that the concentration and flow rates be verified, and these should be checked periodically by a technician on some regular schedule or interval (*e.g.*, monthly). All ozone generated should be accounted for, by checking for leaks in the system and by proper destruction of any excess ozone.

If the ozone is being applied as a gas for the fumigation of a storeroom or cellar, monitoring at the far end of the room and feedback control is desirable. If the ozone is dissolved in water and this water is subsequently used for sanitation, there is always some excess ozone that will not be dissolved into solution.

No ozone mass transfer system is 100% efficient. Excess ozone, or entrained ozone gas, must be "de-gassed" or separated from the water stream prior to delivery to equipment or the processing environment. This excess ozone must also be destroyed or decomposed back to oxygen before being released back into the atmosphere. Thermo-catalytic ozone destruct units are small, efficient, and available for this purpose.

It is not enough to just purchase an ozone generator. Your winery must also have maintenance, verification of performance, monitoring, and, especially in the case of mobile ozone units, an in-place systems approach that ensures the safe use of ozone in the workplace. Properly used, these ozone sanitizing systems are much safer than chemical (chlorine and caustics) or heat-based sanitizing systems.

Oxidation of equipment and facilities

One concern is that use of ozone will oxidize equipment and facilities, and this can happen if the materials are incompatible with ozone. Most materials used in food processing are compatible. Stainless steel (*e.g.*, 316L) is corroded less by ozone than by chlorine, and common plastics used in food processing are generally resistant, including ECTFE (Halar®), PTFE (Teflon®), PVDF (Kynar®), PVC (rigid, schedule 80 or 40), and silicon tubing and gaskets. Natural rubber will readily degrade; however FPM (Viton®) and Teflon gaskets are very stable.

When ozone is used in high concentrations, stainless steel, Teflon, and Kynar are the best construction materials. PVC should be avoided under high concentration conditions. In general however, high concentrations (in the low percent range) are only found inside the generator or in the ozone-to-water contacting system. Aside from natural rubber material, brass and copper should also be avoided for concentrations over 1.0 ppm of ozone dissolved in water.

Evaluating ozone sanitation

Recently, in my laboratory at California Polytechnic State University, a study was performed to evaluate ozone's effectiveness as an environmental sanitizer. The fruit and vegetable pilot plant in the university's Food Science and Nutrition Department became the location for the test, and the ozone system used in the study was a DEL Industries model AGW-0500 Surface Sanitizer™. This system is able to deliver an applied dose of 2.0 ppm through a 10 gpm hand-held spray wand, typically delivering a residual (measurable) dose of around 1.5 ppm ozone-in-water solution. Environmental ozone monitoring was performed using an EcoSensor ambient monitor, and concentrations in solution were verified using a Rosemount Analytical dissolved ozone monitor (model 1054B).

Various surfaces in the facility were sprayed with the ozonated water in a back and forth fashion for one minute. The test surfaces included a polished stainless steel mixing kettle and table top, stainless steel shroud, central floor drain, a plastic shipping container, and two locations on the non-slip epoxy-coated concrete floor of the facility (area 1 is high-traffic and area 2 is low-traffic).

Test areas were not cleaned prior to sanitation, so only the effect of the ozone spray wash was measured. Testing was repeated four times, and microbial load of a 100 cm^2 area was measured before and after ozone application, using both aerobic plate count (3M APC Petrifilm) and bioluminescence (Biotrace; UniLite UXL 100 Bioluminometer). Results are presented in Table I.

Table 1

Location	% Reduction (plate count)	% Reduction (biolum)
Stainless steel (kettle)	89.7 to 98.2	87.6 to 91.8
Stainless steel (table top)	98.9 to 99.7	90.0 to 93.8
Stainless steel (shroud)	63.1 to 99.9	68.8 to 92.2
Floor surface, area 1	67.0 to 95.6	75.2 to 96.1
Floor surface, area 2	84.3 to 99.9	32.8 to 48.8
Floor drain	*	54.7 to 66.5
Floor drain (2 minutes)	77.5	92.9 to 99.4
Plasic shipping container	96.9 to 97.2	68.8 to 97.4

*Due to drainage and sampling problems, results from this location were inconclusive.

The results indicate that ozone applied as a spray wash is effective in reducing microbial load in the processing environment. The drain presented problems during the test because the ozonated water applied to the drain washed throughout the long central drain ditch, which made results inconclusive. A second test on the drain for two minutes of exposure did provide a reduction in

One advantage ozone has is its ability to readily oxidize microbes in solution. Thus, once a surface is spray-washed, the microorganisms physically lifted from the surface will be killed as they find their way to a drain. The data above represents one series of tests over a two-week period (evaluations performed approximately every third day). With continued or daily use, it is reasonable to expect that the microbial load will be significantly eliminated at all locations exposed to the ozone. Because ozone requires no storage or special handling or mixing considerations, it may be viewed as advantageous over other chemical sanitizers. Some may consider the fact that ozone leaves no sanitizing residual a disadvantage, but if a residual is desired, there are many other sanitizers available to accomplish that. Ozone can be considered a complimentary sanitizing regime to help maintain the overall cleanliness and sanitation of a winery.

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