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Ozonization Technology and Its Effects on The Characteristics and Shelf-life of Some Fresh Foods: A Review

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Abstract

The agricultural products such as vegetables, fruits, meat and liquid based products are vulnerable to physical, chemical and microbiological damage due to their high moisture and organic matter contents. On the other hand, the consumer demands high quality food respectively fresh, clean, healthy, and safe. Ozone may be used an alternative or complementary food cleaning. The effectiveness of ozone against contaminating microorganisms present in agricultural products depends on several factors. Mechanism on ozone's cleaning and sanitizing role in some food products are discussed. Application of ozonisation on cauliflower, red chili and guava crest and liquid based products exposed in some various ozone concentrations and exposures, on microbes' inactivation are also discussed.

Keywords: ozonisation; food properties; shelf-life.

1. Introduction

Food is a basic need for every human being, which its existence has a fundamental role for human right. Humans consume the food to obtain the nutrients they need in order to keep their body health. People should obtain sufficient food with good quality, quantity and safety as well. Food safety indicates that the food consumed should be free from substances that endanger human health. Foods also need to be free from microbiological contamination, chemicals, or other materials, and do not contain any elements that contradict with culture or religions. On the other hand, food commodity is known as a material that can be easily decay. The decay occurs in the form of microbiological quality, irregularities on the material, chemical composition, either its texture or structure. Furthermore, the decay of agricultural products also occur in the form of appearance, colour or flavour ingredient.

The decay of agricultural products is contributed by various factors, such as the growth than activity of microorganisms, insects or rodents, enzyme activity, non-enzimatic chemical reactions, and physical damage. Among those factors, the decay of agricultural products due to the existence of microbes needs serious attention. Preservation efforts are needed to reduce damage of food products, or longer shelf-life as well as aspects of quality and food safety is maintained. Generally, preservation methods only focus on preventing or slowing down the destruction of microorganisms; eliminating or inhibiting the growth and activity of microorganisms by using low- temperatures, drying, using of anaerobic conditions or using chemical preservatives; and killing microorganisms with sterilization or radiation process.

The application of ozone gas is offered as a solution to preserve food in reducing the amount of harmful microbes and chemical contaminants attached to food. Ozone is an anti-microorganism compounds that can be used for handling, storage and processing of food in the form of gases and liquids, including raw materials and products for the fresh-cut fruit and vegetables [1]. Ozone has the abilities to kill microorganisms and eliminate hazardous materials, so it considerably has good prospects as an eco-friendly cleaning materials. In addition, ozone can be used for handling, storage and processing of food in solid and liquid raw materials. Ozone is a powerful oxidizing agent that can be used to kill bacteria (sterilization), eliminating color (decoloration) and odors (deodoration) and describe organic compounds (degradation). Ozone has been used as a cleaner alternative in the processing of vegetables and fruits, even it can be used before harvesting [2]. Ozone reacts with contaminants either directly as ozone molecules (O_3) or indirectly as derivatives ozone free radicals such as OH and H_2O [3]. Khadre *et al.* [4] stated, the inactivation of bacteria is complicated due to ozone attacking various primary cells, proteins, unsaturated lipids, enzymes and nucleic acids in the cytoplasm, as well as protein and peptidoglycans in coatings of spores and virus capsids. The next chapter, we will discuss the application of ozone on vegetables, fruits, meats and some liquid based foods. Moreover, preservation mechanism, as well as its impact on food quality.

2. Methods of ozone generation for food industry

Ozone is formed by a high energy input splitting the O_2 (oxygen) molecule. Single O rapidly combines with available O_2 to form the very reactive O_3. In nature, ozone is formed by UV irradiation (185 nm) from the sun and during lightning discharge [14]. Commercially, UV-based generators pass ambient air (20% O2) or oxygen-enriched air across an UV light source, typically less than 210nm. These systems have a lower cost but also have a more limited output than corona discharge systems. Corona discharge generators pass dry O_2 enriched air or highly purified oxygen across a high electric

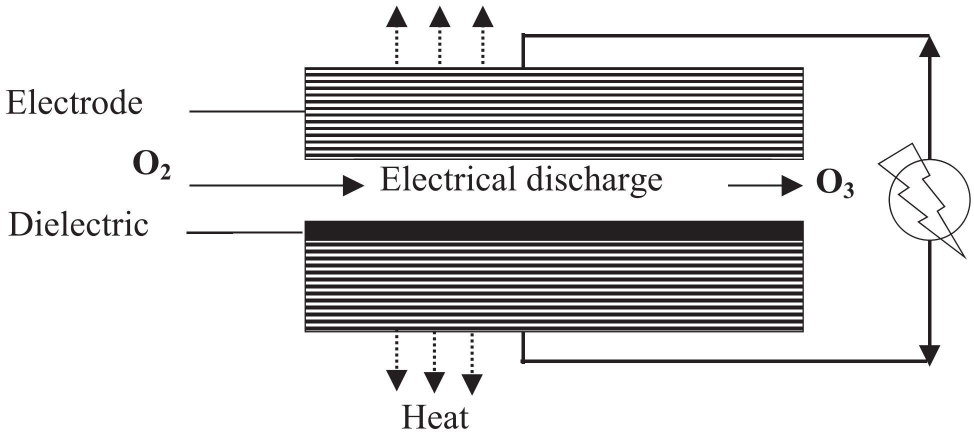

Figure 1: Corona Discharge Cell Configuration [2].

voltage (>5,000V) or corona (Figure 1). Ozone then pumped into a contained postharvest air volume or pulled into a water stream system as already developed by Setiasih *et al* [5], water containing ozone then called ozonized water (Figure 2). Ozonized water could be used as disinfectant through washing process for many agricultural commodities such as chilli, cauliflower and guava. Some of those commodities will be discussed in this review.

Ozone could introduce to the water base food system by gas bubbling method. In a bubble column ozone gas interacts with liquid food, where ozone is consumed followed by a chemical reaction involving oxidation. The overall reaction rate is governed by two steps (1) the mass transfer from the gas phase to the liquid phase and (2) the chemical reaction in the liquid phase [6]. Excess of ozone that not dispersed in water could be captured and destroyed to prevent corrosion. One method of destruction is by UV light at a longer wavelength, 254 nm, combined with the use of a catalytic agent or granular activated charcoal.

3. Ozone and its properties

Ozones a very pungent, naturally-occurring gas with strong (highly reactive) oxidizing properties. Ozone has a very long history of safe use in disinfection of municipal water, process water, bottled drinking water, and swimming pools. More recent applications include treatment of wastewater, dairy and swine effluent, cooling towers, hospital water systems and equipment, aquariums and aquaculture, water theme parks, and public and in-home spas. In clean, potable water free of organic debris and soil particulates, ozone is a highly effective sanitizer at concentrations of 0.5 to 2 ppm (1 mg/L = 1 ppm) [2].

Ozone is almost insoluble in water (0.00003g/100mL) at 20°C and effective dispersal is essential for antimicrobial activity. Ozone's disinfectant activity is only marginally

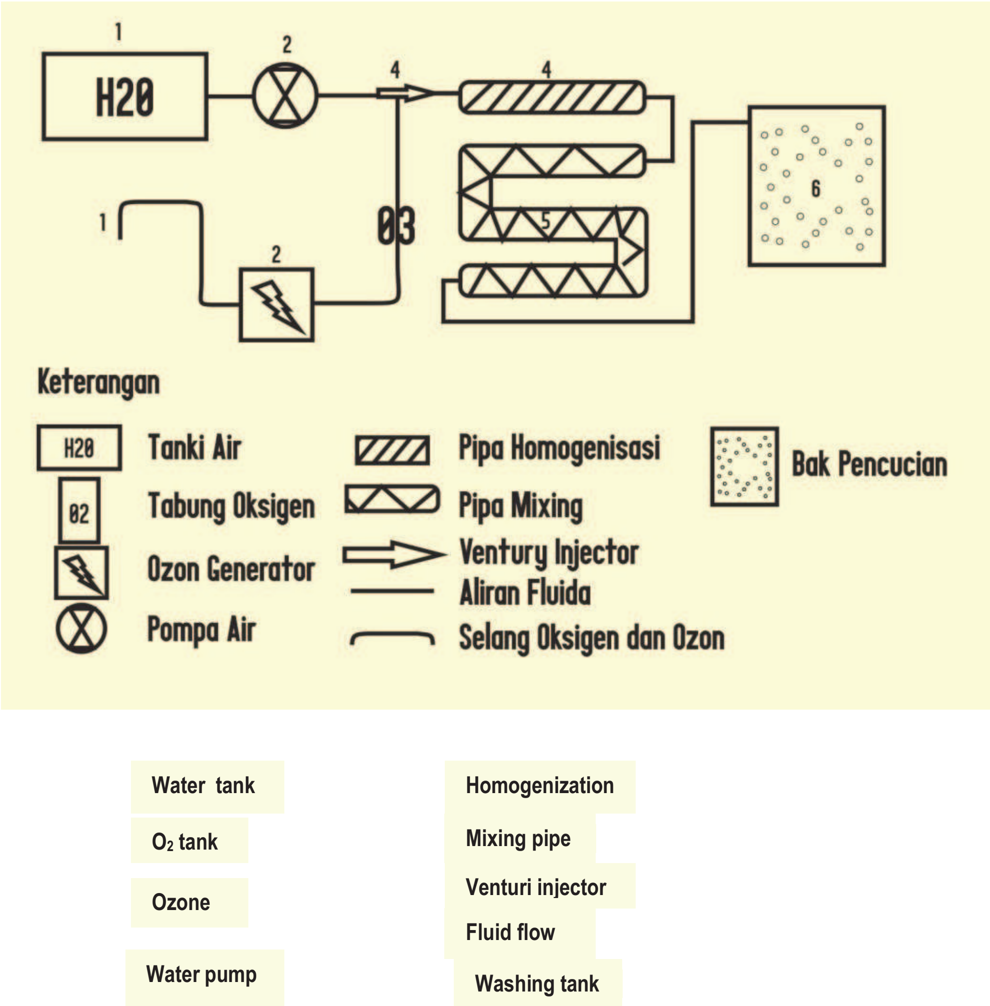

Figure 2: Schematic diagram of the ozonizer TIP-01 [5].

affected at a water pH from 6 to 8.5. Ozone is highly corrosive to equipment and lethal to humans with prolonged exposure at concentrations above 4 ppm. Ozone is readily detectable by human smell at 0.01 to 0.04 ppm. The limits of ozone continues exposure is 0.1 ppm during an 8h period and 0.3 ppm for a 15 min period. At 1 ppm ozone has a pungent disagreeable odor and is irritating to eyes and throat [8]. Ozone is also highly unstable in water and decomposes to oxygen in a very short time. Less than half the activity remains after 20 minutes in pure water and may only have a residual of 2 to 3 minutes in more complex, potable water. In postharvest packing water or fresh cut processing water, with suspended soil and organic matter, the half-life of ozone activity may be less than one minute. Lower water temperatures extend the half-life of ozone. Specific water quality constituents; increasing alkalinity, soluble iron and manganese content, hydrogen sulfide, humic acids, and soluble organic compounds will delay the build-up of detectable ozone residuals in the water and reduce the apparent half-life of ozone. As a consequence of the low stability, maintaining effective concentrations of dissolved ozone for microbial disinfection by using remote ozone generation and injection into a centralized water system, as is done with chlorine and chlorine dioxide, has proved difficult or impractical. This low stability, however, is one of the perceived benefits of ozone as a disinfectant [8]. The effectiveness of ozone to reduce the number microorganisms is influenced by several factors such as temperature and ozone concentration. The lower the temperature the higher the solubility of ozone in the material so that have more time to kill microorganisms. This means that the longer the contact with ozone material, the more the concentration of dissolved ozone and chance to kill micro organisms [9].

4. Safety of ozone

The application of ozone in the food processing specially as an antimicrobial agent in the direct contact has approved by the U.S. Food and Drug Administration (FDA). Ozone is oxidizing agent which is effective against and reduce pathogen microorganisms at low concentrations and short time contact. Ozonation process has no hazardous residues on food product after contact ozone and food products. Meanwhile, in the process, ozone is a toxic gas and potent to effect the illnes if inhaled in the high concentrations. Toxicity symptoms including sharp irritation of nose and throat for the ozone concentration 1-2 ppm and 3-6 h exposure time. Ozone level more than 50 ppm will be fatal effect to the human body [10].

For commercial use, ozone must be produced on site and it is classified as GRAS (generally recognized as safe) for food contact applications in the USA. The product of ozone degradation is oxygen; therefore, it leaves no residues on treated commodities. There are other conceivable benefits to ozone, such as depuration of mycotoxins [9].

5. Ozone Application In Food Industry

5.1. Ozone treatment on vegetables and fruits

Traditionally, ozone treatment within the fruit and vegetable processing industry has been carried out for surface decontamination of whole fruits and vegetables by either gaseous treatment or washing with ozone-containing water. Several studies have been reported relating to the evaluation of ozonation treatment to fruits and vegetables. Barth *et al.* [11] evaluated ozone exposure for prevention of fungal decay on thornless blackberries. The fruit was harvested and stored for 12 days at 2°C in 0.0, 0.1 and 0.3 ppm ozone, then evaluated for fungal decay (*Botrytis cinerea*), anthocyanines, color and peroxidase activity. The result showed that ozone storage could suppress fungal development for 12 days, while 20% of control fruits showed decay. The treated fruit did not show observable injury of defects. Meanwhile Kondo *et al.*

[12] reported greater than 90% reduction of total bacterial counts upon treatment of Chinese cabbages with ozonated water (2.3 mg/L) for 60 minutes. Whangchai, Saengnil, Uthaibutra [13] observed the effect of ozone on the surface microorganism population on longan and postharvest decay. They reported that fumigation with ozone at 200 μL. L^{-1} effectively inhibited growth of *Lasiodiplodia* sp and completely inhibited growth of *Cladosporium sp.* Longan fruit exposed to ozone for 60 min had the greatest reduction in microorganism population. In another fruit, Botondi *et al.* [14] reported that ozone can reduced fungi and yeast in wine grapes by 50%. Shock ozone fumigation (1.5 g/h) for 18 h before grapes dehydration can be used to reduce the microbial count during dehydration without affecting polyphenol and carotenoid contents. Ozone treatment to preserve vegetable reported by Musaddad [13], that submersion of cauliflower fresh-cut in normal water (without ozone) could reduce microorganisms of 0.75 log CFU/g. The use of ozone water with 0.5 ppm and 1.0 ppm are resulting more reduction in total microorganisms, which were 1.08 log CFU/g and 1.09 log CFU/g. Meanwhile, research conducted by Rodger *et al.* [16] was using ozone water with initial concentration of 1.3 ppm. After the lettuce was included, ozone was blown to the bowl for 3 minutes, resulting in 1.2 log CFU/g mesophyllic microorganisms and 1.8 log CFU/g psychotropical microorganisms deactivated. Meanwhile application of ozonation on red chilli exposed in some various ozone concentration and time of treatment were reported by Setiasih et al. [5]. The treatment used was red peppers that were not immersed in water and red chili peppers immersed in ozonized water at 1.9 ppm for 5 minutes. Results showed that immersion in ozonized water able to reduce13.35% of weight loss, 9.64% of totally microorganisms, 14.44% of hardness changes rate, 51.42% of pesticides, did not affect decline rate of vitamin C, and could maintain the brightness of color, moisture content and organoleptic characteristics (color, texture and overall appearance).

Ozone destroys microorganisms by oxidation of cellular components such as sulfhydryl groups of amino acids in enzymes and oxidation of cell membrane. Longer exposure to ozone damages the cellular membrane. Fungal hyphae penetrate into the fruit through lenticles, making fruit more susceptible to decay [26]. Study conducted by Karaca and Veliough [17] showed that mango immersed in ozonized water increased in reducting sugars, total dissolved solids and acidity with longer of immersion times. Meanwhile vitamin C content, physical characteristics such as color, flavor and tenderness decreased with the length of immersion time. The changes might be attributable to the ability of ozone to oxidize vitamin C. However, it revealed different results and showing that immersion of mango in ozonized water resulted in higher values of total acidity, total soluble carbohydrates, vitamin C and flavonoid contents. The authors argued that ozonation could promote a stress leading to higher respiration rate, thereby the augment in the solubilization of polysaccharide reserves. Ozonization might also trigger injury stress to the cell leading to increased vitamin C content. It might also be referred to the ozon inhibitory effect on the enzyme responsible for vitamin C degradation.

The effect of ozone on phisico-chemical characteristics is apparently dose dependent. It is obvious from the study on papaya by Ong *et al*. [18] that ozone fumigation as much as 5 ppm was able to lower the respiration rate and delay ripening as compared to the control. Higher ozone concentrations however lead to more ethylene production and tissue injury. This means proper levels of ozone is needed to trigger antioxidative defense mechanism and extend shelf life of fruits.The sensitivity to ozon treatment also seemed to differ from one type of fruit to another. Study on fresh-cut pineapple, banana 'pisang mas' and guava showed different effect in terms of vitamin C, the total phenol and flavonoids. The exposure to the gaseous ozone was able to increase the polyphenol and total flavonoid content for the treatment as long as 20 min for pineapple and banana, while it was around 10 minutes for the increase in the flavonoid content of guava. The increase in the total phenol and flavonoid in those fruits with the increase in treatment time could however compromise the vitamin C content which showed the decreased content with the increase treatment time. To amplify the benefit of ozone treatment, it is advantageous to combine the treatment with another one as Whangchai *et al*. [13] did by combining the treatment with organic acid. It was found that longan fruit treated with ozone in combination with either oxalic or citric acid had less browning and a reduction of polyphenol oxidase.

5.2. Ozone treatment on meat products

Beef carcasses have the potential to become contaminated with faecal material through the normal dressing procedure. Because these bacteria may include pathogens, it is of interest to apply antimicrobial treatments, like ozonation to beef carcasses in order to remove any faecal contamination and pathogens that might be present. Greer and Jones [19] evaluated the impact of gaseous ozone treatment on beef carcass bacterial spoilage profiles and onmeat quality and carcass shrinkage. They placed one beef carcass paired side into a cooler supplied with continuous ozone generation and compared the findings to conventional chilling. They found that psychrotrophic bacterial growth was retarded on carcass surfaces while under ozone atmosphere. However, carcass treatment with ozone atmosphere did not reduce bacterial growth on steaks from these carcasses. The reduction of microbial pathogens is important on carcass and tissue surfaces in order to improve the safety of intact, whole muscle cuts. However, the reduction of microbial pathogens on beef trimmings destined for ground beef is also important and is very challenging. This is because any contamination that might occur on beef tissue surfaces will become inoculated into the interior of ground beef when it is ground.

Stivarius *et al.* [20] evaluated the impact of ozone at differing exposure levels and of chlorine dioxide as beef trimming treatments for their effecton ground beef microbial characteristics. For the study, they inoculated beef with *E. coli* and *S. typhimurium*. The beef were then treated with 1% ozonated water for 7 minutes or15 minutes, or with 200 ppm chlorine dioxide, and compared with a control. Ground beef was then produced from the beef, packaged and placed into simulated retail displays for up to 7 days. *E. coli*, *S. typhimurium*, coliform and aerobic plate counts were carried out on the ground beef during display. Results showed that the 15 minute ozone treatment of beef was effective for reducing all microorganism counts measured in ground beef, while the 7-minute ozone treatment reduced aerobic plate counts and *S. typhimurium* counts significantly.

Ozone has been used as a pretreatment before cooking to determine any synergistic activity on reducing microorganisms. Novak and Yuan [21] treated beef surfaces with ozone followed by cooking at 45–75 °C The authors reported a 1-2 log CFU/greduction in enterotoxin producing strains of *C. perfringens*. Moreover, a reduction in spore count was also observed, but only a very low reduction was was obtained, indicating that spores were much more resistant to ozone than thermal treatment. The authors concluded that ozone treatment followed by heat treatment allowed reductions at cooking temperatures that normally would not impart the reduction by themselves. Ozone has also been evaluated for its effectiveness against *L. Monocytogenes*in ready-to-eat products such as ham. Jhala *et al.* [22] reported on the impact of ozone and a bacteriocin from *Propionibacterium shermanii* on *L. monocytogenes* in ready-to-eat cooked and cured ham. They reported a synergistic activity between ozone (0.2–1.0 ppm) and the bacteriocin, causing an inactivation of up to 3 log reductions of *L. monocytogenes*. Ozone has been shown to reduce microorganism levels on red meatcarcasses and cuts. Although it is likely there are a number of mechanisms involved in the mode of action of ozone against microflora, one is the oxidation potential of ozone. Ozone has a high oxidation potential (−2.07 V) and consequently poses some unique challenges to retaining meat quality. Specifically, oxidation in meat systems can influence meat colour and lipid stability characteristics. Oxymyoglobin is the pigment principally responsible for the redness of meat. When subjected to oxidation potentials, this pigment can revert to the oxidised pigment form, metmyoglobin, which is brown in colour. Because consumers often associate freshness with red colouration, the development of metmyoglobin or brown colour due to pigment oxidation may affect consumer acceptance of the ozone-treated products [20]. In addition to causing negative effects on meat pigment chemistry, oxidation can also have an impact on lipid stability. Oxidation of lipids can lead to adverse quality attributes in meat. And because ozone is a strong oxidiser, this can pose concern for meat quality in products treated with ozone if care is not taken.

6. Ozone treatment on liquid based foods

Ozone is also applied to ensure the safety of food products including some liquid based foods such as milk and juice where above 80% consist of water. Ozonation in some liquid based foods are influenced by several factors, such as temperature, retention time, ozone concentrations, pH. At high temperature, the resistance and the existence of ozone in the water is reduced. The retention time of ozone in water at a temperature of 25 °C was 15 minutes, while at a temperature of 35 °C is 8 minutes [23]. Retention time of ozone in water is decrease with the increase of water temperature. Meanwhile, water with an acidic pH can inhibit the decomposition of ozone, due to the low pH which indicates high alkalinity, thus can cause ozone retention time increases. Concentration of ozone and its retention time have important role in determining the efficiency of ozonation. Time of ozonation is the amount of time required by ozone to inactivate microorganisms in the medium such as water or liquid based foods. The concentration and time of application is used to calculate the amount of disinfectant needed and to determine the effectiveness of a particular disinfectant in killing microorganisms in liquid food.

The concentration of ozone is used to inactivate microorganisms in the medium of water or liquid based foods is about 0.5 to 4.0mg/L. For E.coli and Streptococcus which are found in milk, at pH 7.0 and temperature 25°-30°C, the ozone concentration of 0.01 mg/L was high enough to inactivate such microorganism. Ozone will react with all protoplasm cells by acting as an oxidant. Ozone will directly attacking the surface layers of bacteria, oxidized the sulfhydryl of the enzyme, or oxidized the lipoprotein and the lipopolysaccharide which are the inner layer of the gram-negative bacteria. This process lead to a breakdown of the cell permeability defense so that the cell becomes paralysis. Only 1% of E. coli and Streptococcus was left after contact with 0.8 mg/L of ozone for 60 seconds [24, 25]. Furthermore, Cavalcante *et al* [26] found that the raw milk studied showed an adequate microbiological quality and the ozonation for 15 minutes was able to causes 0.5 to 1 decimal reductions in the milk native flora. Complementary, no changes were observed in the physicochemical parameters of the milk. Thus, the results highlight the ozonation could used as an alternative method to reduce the microbial load in raw milk, improving its quality and increasing the milk shelf-life before thermal processing. Residues of ozone are toxic to all kind of microorganism, but the ozone easily biodegradable. Ozone only last for a few minutes in water base material so that the residual of ozone is already disappear and harmless to organisms that use them.

7. Conclusion

Every agricultural commodity is unique in both of microbiological and physical chemistry as well as handling practices. Most of food processing operation and facility are unique in the manner by which they wash their raw agricultural commodities prior to further processing or packaging. Ozone aplication has been shown to reduce the number of microbes in some fruits, vegetables, beef, and liquid base products and efective as common sanitizing agents. The methods and ozone concentration used to reduce the number of microbes in agricultural products could vary depending the condition of the medium. Microbial inactivation by ozone is mainly due to the rupture of cellular membranes. However, as ozone only last for a few minutes in the medium, ozone is potential to be applied for sanitizing agent for many kind of agricultural products.

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