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# Ozone Application in Fresh Fruits and Vegetables

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**Abstract –** The demand of fruits and vegetables across the world had increased throughout the years which urge the need to have better and proper way to increase produce safety, quality and postharvest life. Traditionally pesticides or other chemicals had been used to encounter microbes related to postharvest diseases. Over time, consumers are concern towards health effect of consuming those produce treated with chemicals. Ozone is one of the approach that provide both of the needs to deal with pathogenic microbes and also give no harmful residue throughout the process. Several reports had proven that ozone can almost kill or inhibit all pathogenic microbes on treated commodity which promote higher quality and postharvest life during storage. This review focus and summarise the use of ozone in the form of aqueous and gaseous towards fresh produces, its benefits and also the precaution during ozone application.

**Keywords:** Disease, postharvest life, quality, safety

## Introduction

The growing demand of foods around the world nowadays had led to increasing need to export and import trade of food especially fresh produce. At the same time, this also led to an increase problem of foodborne illness related to fresh produce which require better storage to prolong its postharvest life as these produces are perishable (Mahajan et al., 2014). The most common cause of fruits and vegetables deterioration is due to microbial contamination, either infection at farm, during or after harvesting. Normally cleaning with tap water or chlorinated water being the most popular choices of cleaning fruits or vegetables. Although this methods are the easiest and well known, recent study had showed that cleaning fresh produce with water only does not reduce any possible contamination especially microbes, while cleaning with chlorinated water may have significant effect against various types of microbes (Suslow, 2000). However, it may not be effective against some pathogenic microorganisms and viruses on fruits and vegetables (Kim et al., 1998; Xu, 1999). Furthermore, trihalomethanes formation from the reaction of chlorine application towards soluble organic compounds can give threat towards human dietary safety as well as environmental pollution (Karaca and Velioglu, 2007). Maintaining the quality and extending postharvest life of product are the most important in fruits and vegetables industry. To overcome this, lots of method have been used which include fungicides, starting from farm until postharvest treatment. However, it has been reported that fungicides may left some residue and toxicity upon application that may cause some health problems and also towards environment. Furthermore, application of chemicals sometimes led to an increase resistant of fungi towards fungicides that being used (Somasundaram et al., 1990). Thus, more potent and toxic fungicides need to be used over time which led to more severe problem regarding chemicals contamination. As consequences, this lead to harmful by products especially towards health issue had led to an increase demand of an alternative safer and potent method (Ikeura et al., 2011). One of the potential treatment method for fresh produce especially for fruits and vegetables is the use of ozone. Due to its high oxidative properties that can kill most microbes, ozone is one of the potential alternatives that can be used as a sanitizing agent for postharvest application. Compared to other gases that are used in postharvest treatment, ozone is generally easy to detect by distinct smell. Previously, ozone is well known for their highly antimicrobial activity and used to disinfect drinking water (Seydim et al., 2004) and lots of other early use of ozone such as disinfection of municipal water, swimming pools (Suslow, 2004) and in cleaning advance wastewater treatment (Rice and Graham, 2001). Now it becomes more common in food industry where it is used as "sanitizer" to treat agriculture product against various kind of microbes (Alencar et al., 2014; Palou et al., 2002; Whangchai et al., 2006). Besides that, aqueous ozone also can be used as fungicides removal towards fruits and vegetables products and are commercially used nowadays in modern home kitchen appliances. An application of 1 to 2 µg/mL aqueous ozone has been reported to successfully remove pesticide from lettuce, cherry tomato and strawberries (Ikeura et al., 2011). Ozone had become an interest treatment in agriculture since it had been recognized as generally recognized as safe (GRAS) (Rice and Graham, 2001). It had become more fascinating since it does not left any residue on the treated product even though it is applied on fresh cut or processed fruits (Sharma et al., 2003) and thus make it suitable for the treated product to be marketed as an organic product. Recent work had indicated that ozone is effective against fungal of postharvest disease while at the same time it helps to increase the shelf life of produce by sanitizing the surface of the product (Alencar et al., 2014; Whangchai et al., 2006). Generally, ozone had been used in postharvest treatments either as treatment prior to storage or during storage (either as one time application or continuous application throughout the storage period) (Palou et al., 2002; Rice, 2010; Skog and Chu, 2001) but no continuous aqueous ozone treatment had been reported.

**Properties of ozone**

Ozone is a natural gas on earth. Naturally, it is produced by reaction of oxygen with ultraviolet light from sunlight or lightning (Suslow, 2004) in which most of these natural ozone gases are on the stratosphere (Anon, 2015). In lower atmosphere, it is formed by oxidation of hydrocarbon mainly from vehicle, electrical transformer and devices (Xu, 1999). Ozone is formed when three molecules of oxygen are combined together. Unlike oxygen, ozone is very oxidative at high concentration and it is also easily decompose back to oxygen in a very short time leaving only oxygen and no other harmful by product (Palou et al., 2006). Ozone has a relative molecular mass of 48 and has density of 2.14 g/L at 0 $^{o}$C and 101.3 kPa and a boiling point of -112 $^{o}$C while having an oxidative potential of 2.07 eV which is very potent oxidizing agent (Palou et al., 2006). It is listed as second most powerful gas with oxidative potential after fluoride (Seydim et al., 2004). High concentration of ozone have deleterious effect towards health, so proper protection need to be applied when dealing with high ozone concentration. With prolong concentration of 4 µL/L, ozone is reported to be lethal to human (Suslow, 2004). The Federal Occupation Safety and Health Administration (OSHA) had set limits for safety of workers to work with ozone. It had been suggested that one could only be exposed to 0.1 µL/L threshold of ozone for only 8 h, while higher concentration of 0.3 µL/L for 15 min.

In commercial application, ozone is produced either by ultraviolet irradiation at 185 nm or by using carona discharge method (Karaca and Velioglu, 2007; Seydim et al., 2004; Xu, 1999) with two types of feed gas either air or pure oxygen (Xu, 1999). In some cases, ozone feed air are filtered to avoid producing other reaction during the process of ozone generation (Tzortzakis et al., 2008). Ozone gas are easily detectable by distinct smell, it has a detectable pungent smell when the concentration is high but it is already detectable by human at concentration as low as 0.01 µL/L (Xu, 1999). In term of oxidizing power, it has been reported that ozone is 1.5 times more potent oxidizing agent compared to chlorine and 3000 times of hypochlorus acid (Suslow, 1998; Xu, 1999).

**Ozone application methods in postharvest**

When ozone is used in postharvest treatment, it can be applied in two forms; i.e. gaseous (ozone in air) or aqueous (ozone in water). Normally units that are used for gaseous or aqueous ozone is parts per million (ppm). The only different is that in air ppm is calculated in volume/volume (µL/L) while in aqueous form it is calculated in weight/volume (µg/mL). In term of molecule comparison, with the same concentration of 1 ppm ozone, 1 L aqueous ozone and 1 L gaseous ozone has relatively different molecules concentration which in favour of aqueous ozone with ratio of 5000,000 : 1 (Smilanick, 2003). While Rice (1986), reported that aqueous ozone have 13 times higher solubility compared to gas ozone below 30 $^{o}$C. The application of ozone as air or aqueous is mostly depend on the fruits or vegetables to be treated. Commodities that are susceptible towards water such as strawberries and mushroom should avoid aqueous ozone and be treated with gas ozone.

*Ozone in air*
Ozone in air is applied in form of fumigation. Normally a chamber is used to contain the gas in order to achieve the desired concentration. Ozone in air is detectable in low concentration as low as 0.01-0.05 µL/L and is described of having smell such as fresh air after rain (Suslow, 1998). Ozone in air has better stability compared to ozone in water (Rice, 1986) and can still be increased by increasing humidity in the applied chamber (Olmez and Dogan, 2002).

In commercial application of postharvest, ozone generator is fixed into a storage room or container (Palou et al., 2002) in order for the treatment to be applied. An air ozone sensor is used to determine the concentration and is fixed inside the contained ozone gas. Some system may come with an automatic switch, in which when the sensor detect the desired concentration is achieved it can automatically kill the ozone generator switch and continue to generate ozone when the concentration is outside the desired range. Meanwhile, certain ozone treatment system might not include ozone sensor within storage facility, instead they use gas sampling method with pump that include an ozone detector (Whangchai et al., 2006). A fan may be included in a system to regulate air for even contact with fruits or vegetables (Ong et al., 2013) while ozone scrubber or potassium iodide are most likely to be placed on the exhaust valve for workers safety (Wu et al., 2007).

*Ozone in water*
Ozone in water is generally the same as ozone in air but the gas were applied into water through tube channel which is bubbled into a container of water. It can be used to replace chlorinated water in order to disinfect the surface of fruits and vegetables from microbes or even just to cool down the produce temperature. The used water can be recycled few times with addition of filtration to get full use of water and potential of ozone oxidation (Xu, 1999).

Compared to gaseous ozone, aqueous ozone is very unstable and oxidized quickly to oxygen in a very short time. Unlike fumigated ozone gas, aqueous ozone are more susceptible towards dirt, organic materials, or soil which can dramatically decrease the ozone concentration in water and thus reducing its oxidation potency (Hill and Rice, 1982). Aqueous ozone must be supplied continuously with new generated ozone gas to maintain the desired concentration. It has been reported that less than half ozone activity remains after 20 min in pure water at room temperature (Suslow, 2004; Xu, 1999). It is known that low water temperature can increase aqueous ozone concentration and delay the half life of ozone activity (Xu, 1999). In theory, the maximum solubility of ozone in water is 29.9 µg/mL at 20 $^{o}$C (Smilanick et al., 1999), but honestly speaking it is not easy to achieve that concentration on normal system. It has been reported by Palou et al. (2006), that solubility of aqueous ozone is affected by many factors such as temperature, pH and pressure, while it solubility only about 0.2 µg/mL at 20 $^{o}$C which is very low, even though it is used at the same temperature as previously reported. Table 1 is the list of temperature and the solubility of water that studied by Rice et al. (1981), which show the relation of temperature towards the solubility of ozone in water. The study indicated that lower water temperature tend to give higher solubility of ozone in water.

*Table 1*: Ozone solubility in water on different temperature $^{o}$C

| **Temperature $^{o}$C** | **Solubility (litre ozone/ litre water)** |
|---|---|
| 0 | 0.64 |
| 15 | 0.456 |
| 27 | 0.270 |
| 40 | 0.112 |
| 60 | 0.000 |

**Effect of ozone towards treated fruits and vegetables**

Since ozone will naturally decomposed onto oxygen, there is actually no harmful effect of using ozone towards food products. As early in 19th century, ozone has been reviewed for its safety towards food products and had since be declared as GRAS for food contact application (Smilanick, 2003). Later in 1990's ozone was declared safe to be used in Japan, France and Australia (Smilanick et al., 1999). In 2003, ozone had received formal approval from U.S FDA to be used as food addictive (Karaca and Velioglu, 2007). The approval of ozone as GRAS and the fact that it does not cause any carcinogenic effect towards human health (Xu, 1999), had increased many parts of the world to use ozone in their food treatment. In Cuba, ozone were used to control fungi that cause papaya rot and also reported to delay papaya ripening (Bataller et al., 2012). While in Davis, ozone had been reported to delay the green and blue mold symptom on citrus for about one week (Palou et al., 2001). Besides, the concern of pesticides residual on fruits and vegetables can also be reduced due to the fact that aqueous ozone has the capability to remove pesticides, chemical residue and toxic organic compounds (Ikeura et al., 2011; Langlais et al., 1991; Wu et al., 2007). Furthermore, ozone is also widely used in treatment for drinking water. It is used to eliminate iron, manganese and sulfur as well as to control the odor and taste of water with additional effect to kill microorganisms (Xu, 1999).

Application of ozone in gaseous or aqueous form have their own advantage and disadvantage. In most cases it depends on the sensitivity of the commodity towards water and the concentration of ozone applied. A specific concentration of ozone must be applied in order to get the desired result. A too high ozone concentration will cause deleterious effect towards fruits and vegetables, while a too low concentration will not give any significant effect on the treated fruits and vegetables. Ozone concentration of 0.4 µL/L has been reported to reduce floret opening on broccoli (Skog and Chu, 2001). While in some commodity like button mushroom (*Agaricus bisphporus*) that treated with same concentration of ozone showed phytotoxicity (Skog and Chu, 2001).

The use of ozone has been reported to have potential in extending the storage life of fresh produce (Rice et al., 1982). Ozone in postharvest application are currently growing. Lots of research on ozone application had been done especially on major exported fruits. It is reported that a right dose of ozone application can delay the ripening of certain kind of fruits and vegetables. Ethylene sensitive commodity such as broccoli and seedless cucumber shelf life were extended in application of ozone at 0.04 µL/L with 3 $^{o}$C (Skog and Chu, 2001) while 10 µL/L ozone concentration can dramatically decrease mango (*Mangifera indica* L.) respiration rate (Tran et al., 2013). The reduction in respiration rate indirectly indicates that ozone can also prolong the shelf life of mango. As reported by Bataller et al. (2012) papaya ripening were also delayed by application of 500 mg/m$^3$ ozone for 2 h every day. Application of ozone had also attract interest not only in extending shelf life, but also in reducing the microbial effect on deteriorating fruits. Reports by Xu (1999) stated that right concentration of ozone not only can extend the storage life of fruits but also reducing the microbial count on the surface of treated fruits. Kim et al. (1999) reported that ozone are able to reduce microbial count on vegetables such as lettuce and cabbage after treatment with ozonised water. Storage with low ozone concentration is able to reduce mold and bacteria significantly on the surface of fruits (Rice et al., 1982; Xu, 1999). Meanwhile, application of 0.3 µL/L ozone towards blackberries can significantly halt fungal development with no physiological injuries (Barth et al., 1995). All of these ozone treatments does not left any harmful residue as ozone only decomposed into oxygen gas. Besides, use of ozone was reported to have significant effect in maintaining quality as well as extending the storage life of fruits and vegetables. Researches that support this finding include Ali et al. (2014) whom reported ozone can maintain the storage life of papaya, while grey mold on table grapes can be reduced by application of 1-2 h of ozone (Gabler et al., 2010) and decay by various pathogen on citrus can be reduced (Karaca, 2010). Furthermore, the use of ozone showed significant effect in reducing bacterial count while retaining all other chemical composition and sensory quality of onion (Forney et al., 2000).

Ozone are also effective against *P. italicum* and *P. digitatum* as reported by Karaca (2010) that synergistic effect of both aqueous and gaseous ozone treatment can inhibit the mycelia growth of *P. italicum* and *P. digitatum* on citrus. Ozone is also significantly better in reducing aging and weight loss of orange compared to the non-treated orange (Karaca, 2010). Ozone can also destroy ethylene gas which can reduce the rate of senescence in ethylene sensitive commodities in mix-stored room. As reported by Skog and Chu (2001) ozone concentration of 0.4 µL/L was effective in reducing ethylene concentration in a storage room of fruits and vegetables where there is an ethylene sensitive crops. However, excess exposure of ozone had been reported to cause physiological injury towards citrus due to high oxidative stress (Karaca, 2010). In some cases, even high ozone concentration that are dangerous to human does not give any significant results. An application of 10 µL/L ozone for 20 min exposure time on citrus does not reduce the incidence of green mold and sour rot cause by *Geotrichum citri-aurantii* and *Penicilium digitatum* (Smilanick et al., 2002). Karaca (2010) also discovered the same as he applied ozone towards green and blue mold (*Penicillium italicum and Penicillium digitatum*) on citrus fruits but discovered there is no significant effect as compared to the non-treated fruits. Since ozone can reduce the odor or foul smell, fruits volatile compounds are also included. It has been reported that aroma of strawberries was reduced in treatment with ozone (Pérez et al., 1999). Weight loss upon continuous ozone treatment had also been reported. Treatment of 0.15 µL/L ozone for 15 days was reported to cause electrolytes release on fruits that indicate damage on cuticle which promote water loss (Rao et al., 2000). While an exposure of 0.3 µL/L of gaseous ozone towards peaches at 5 °C and 90% RH had caused significant water loss compared to non-ozonated peaches but similar exposure and storage condition towards Flame seedless table grapes did not cause any significant water loss (Palou et al., 2002). This proved that different fruits have different response towards ozone and further research need to be done in order to determine the best treatment for each fruits.

## Conclusion

Despite all of the good things ozone can do, some caution must be taken before considering any treatment. Concentration, time of exposure, sensitivity of the commodity towards ozone, worker's safety and cost are some of the things that are worth to be considered. Too high exposure of ozone can have deleterious effect not only towards human health but also to fruits and vegetables. Commodity with good aroma should be avoid since ozone can "neutralize" the volatile compound of it. Moreover, the end product of reaction of ethylene destruction by ozone is not well known. Furthermore, the fact that ozone are naturally decomposed into oxygen which can contribute higher respiration rate of oxygen sensitive commodity need to be considered before it is applied commercially.

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